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ORIGINAL ARTICLES

NEW EXPERIMENTS WITH VAUGHAN'S CRUDE SOLUBLE POISON*

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THE toxicity of Vaughan's Crude Soluble Poison is of great interest from the viewpoint of protein chemistry and its applications to the problems of serology. Irrespective of interpretations advances in our knowledge relative to either the chemistry of this remarkable preparation or its actions and transformations within the body are certain to lead to results pregnant with possible applications.

The present paper is the outcome of a series of observations, the continuation of studies carried through in this laboratory for a number of years. The particular problems which form the basis of this communication are in the first place the determination whether the results already published with respect to the toxicity of Vaughan's Crude Soluble Poison with the guinea pig as subject may be applied to the rat, and further modification of the preparation of the poison together with closer inspection of the fractions which accompany the poison.

PREPARATIONS

The preparations which are pertinent at present are as follows:

Preparation A.—Made from casein exactly according to Vaughan's original method except that the alkaline alcohol extract was allowed to cool before filtration. It will be recalled that in the original method the period of heating was one hour twice repeated with fresh portions of alkaline alcohol.

Preparation B.—Made from casein exactly as Preparation A except that the period of heating for each extraction was two hours instead of one hour.

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Preparation D.—The method given for Preparation A was exactly followed with coagulated egg white.

Preparation F.—Vaughan's nontoxic residue from the preparation of the poison from casein was treated as in the preparation of Preparation A.

Preparation G.—Made exactly as Preparation A except that the alkaline alcohol mixture was filtered hot instead of filtering after cooling.

These preparations served as the starting point for the determination of the toxicity experiments. Other preparations employed will be explained in connection with the individual substances above.

OBSERVATIONS*

Preparation A.—From the data presented in Tables I and II it may be observed that Vaughan's Crude Soluble Poison possesses for the rat a toxicity

TABLE I

PREPARATION A. VAUGHAN'S CRUDE SOLUBLE POISON
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 3, 1922.

RAT*	WT.	DOSE PER 100 GMS.	INJECTED
	gms.	mgms.	P. M.
1	245	10	2:39
2	330	10	2:40
3	270	15	2:40
4	300	15	2:40
5	260	20	2:41
6	150	20	2:41
7	250	25	2:42
8	250	25	2:43
9	215	30	2:44
10	295	35	2:45

At 3:00 P. M. all animals seemed quiet and indisposed to move or be moved.

*All recovered in 2 hours.

TABLE II

PREPARATION A. VAUGHAN'S CRUDE SOLUBLE POISON
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 6, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	165	25	3:07		1
2	240	25	3:08		1
3	245	50	3:10	29 min.	
4	270	50	3:09	53 "	
5	265	100	3:11	47 "	
6	125	100	3:13	26 "	
7	165	125	3:14	23 "	
8	120	125	3:15	20 "	
9	115	150	3:16	16 "	
10	132	150	3:17	15 "	

Death—respiratory with convulsions. Nose, feet and ears became cyanotic.
Autopsy—few intestinal hemorrhages. Lungs collapsed, seemed normal.

*The rats employed were obtained from the experimental colony of Osborne and Mendel. Generally the substances injected were dissolved in physiological salt solution or water without changing the reaction of the substance. When insoluble in these solutions a faintly alkaline solution was employed.

which is quite comparable with that for the guinea pig. The rat may therefore be employed in experimentation with this substance. In doses up to 25 mg. per 100 gm. of rat this particular preparation of Vaughan's Crude Soluble Poison was nonlethal. It was rapidly lethal, however, in doses of 50 mg. per 100 gm. of rat and above, death occurring more rapidly with increase of dosage.

Preparation A(B).—This substance comprised the HCl + NaCl precipitate forming on neutralization of the alkaline alcohol solution. According to the toxicity figures in Table III it possesses relatively few poisonous properties even in doses as high as 200 mg. per 100 gm. of rat.

Preparation A(C).—This preparation was that portion of the residue from the alcoholic distillation insoluble in absolute alcohol. Little or no toxicity

TABLE III

PREPARATION A(B). THE HCl + NaCl PRECIPITATE FORMED ON NEUTRALIZATION OF THE ALKALINE ALCOHOL SOLUTION

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	140	50	3:44		1
2	105	100	3:44		1
3	140	150	3:45	Found dead 9:30 P. M.	
4	105	200	3:45		1

Animals soon became very prostrated, nose became cyanotic and they were weak on the feet.

TABLE IV

PREPARATION A(C). PORTION OF RESIDUE FROM ALCOHOL DISTILLATION INSOLUBLE IN ABSOLUTE ALCOHOL

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	RECOVERED
	gm.	mg.	P. M.	
1	135	50	3:02	1
2	100	100	3:02	1
3	90	150	3:03	1
4	115	200	3:05	1

Not affected—no symptoms.

is possessed by this substance—see Table IV—even in relatively large doses—200 mg. per 100 gm. of rat.

Preparation A(D).—Portion of A(B) insoluble in absolute alcohol. From Table V it is apparent that large doses of this preparation are without noticeable effect.

Preparation A(E).—Portion of A(B) soluble in absolute alcohol. Doses up to 200 mg. per 100 gm. of rat are nontoxic. See Table VI.

From these observations it is evident that of fractions formed during the preparation of this sample of Vaughan's Crude Soluble Poison none exhibited

evidence of toxic properties except the presumably toxic fraction, namely, Vaughan's Crude Soluble Poison.

Preparation B.—This preparation was made in exact accordance with Vaughan's directions except that the heating was carried through for a period of two hours instead of one hour. The data in Table VII demonstrate that this preparation of Vaughan's Crude Soluble Poison is not as toxic as Preparation A. It is quite possible that the extra period of heating is responsible for this difference although different preparations of this substance formed from equal periods of heating show just as great variations in toxicity as may be seen from Vaughan's investigations and from former communications from this laboratory.

Preparation B(A).—Presumably comparable to Vaughan's nontoxic residue. Very unexpectedly this fraction gave evidence of distinct toxicity being fatal in doses of 100 mg. per 100 gm. of rat (Table VIII). It is quite possible that the longer periods of heating so altered the original substance as to change the solubility of unknown substances to a degree such that they were included in the nontoxic portion. This inclusion of toxic products in the presumably nontoxic residue may account perhaps for the lower toxicity of this preparation (Preparation B) of Vaughan's Crude Soluble Poison.

TABLE V

PREPARATION A(D). PORTION A(B) INSOLUBLE IN ABSOLUTE ALCOHOL
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	RECOVERED
	gm.	mg.	P. M.	
1	130	50	2:48	1
2	75	100	2:49	1
3	110	150	2:50	1
4	100	200	2:50	1

No apparent symptoms. Animals appeared very thirsty.

TABLE VI

PREPARATION A(E). PORTION A(B) SOLUBLE IN ABSOLUTE ALCOHOL
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 30, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	RECOVERED
	gm.	mg.	P. M.	
1	110	50	2:53	1
2	80	100	2:53	1
3	115	200	2:55	1
4	95	200	2:45	1

No apparent symptoms.

Preparation B(W).—The residue from dialyzed HCl + NaCl precipitate. This preparation shows a comparatively low toxicity since no apparent symptoms were to be observed with doses of 150 mg. per 100 gm. of rat. With a dose of 200 mg. one rat died during the night of injection. (See Table IX).

Preparation B(W'').—Portion of B(W) soluble in absolute alcohol. This preparation was devoid of toxic effects even in relatively large doses since

200 mg. per 100 gm. of rat could be injected without apparent symptoms. (See Table X).

In summary, the striking feature of this preparation and its by-products is the comparatively high toxicity of that portion which presumably corresponds to Vaughan's nontoxic residue. The only explanation apparent is that the longer period of heating in its preparation possibly changed either the chemical character of this fraction or its solubility so that it is included in this fraction instead of with the toxic end product. This change in the toxicity of the

TABLE VII

PREPARATION B. VAUGHAN'S CRUDE SOLUBLE POISON
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 4, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	A. M.	
1	225	10	10:46	
2	290	20	10:46	
3	225	30	10:47	
4	215	40	10:47	
5	150	50	10:48	
6	185	60	—	
7	235	70	10:50	3/5/22 Found dead 12:00 noon
8(B)	290	80	—	3/6/22 Found dead 3:00 P. M.
9	290	90	10:53	11:49 A. M.
10	250	100	11:04	11:40 A. M.
11(C)	260	110	11:05	3/6/22 Found dead 3:00 P. M.
12(A)	205	120	11:05	11:14 A. M.
13	180	130	11:06	3/8/22 Found dead
14(B)	295	60	11:07	3/6/22 Found dead 2:00 P. M.
15	240	80	11:08	11:46 A. M.

Animals became sick at once with generalized weakness. They tend to fall to one side as if they had muscular weakness of the legs with occasional spurts of activity. Convulsions are rare.

(A) Autopsy showed abscesses in lungs, otherwise normal.
(B) Diffuse peritonitis, lungs negative.
(C) Diffuse peritonitis with intestinal perforation from Peyer's patch.

TABLE VIII

PREPARATION B(A). VAUGHAN'S NON-TOXIC RESIDUE
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 2, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	130	50	4:04		1
2	75	100	4:05	Found dead 8:30 P. M.	
3	110	150	4:07	Found dead 5:30 P. M.	
4	100	200	4:06	Found dead 8:00 P. M.	

Ears and nose became pale at once. At 5:30 Nos. 2 and 4 were very sick. Breathing was very rapid.

Autopsy showed intestines filled with fluid blood. Vessels of testicles deeply engorged. Liver apparently normal, very few adhesions. Heart normal. Lungs collapsed and pale.

nontoxic residue may account perhaps for the diminished poisonous effect exhibited by this preparation of Vaughan's Crude Soluble Poison.

Preparation D.—Vaughan's Crude Soluble Poison from coagulated egg white. It was of interest to determine the influence of previous heating of the protein upon the toxicity of the end product formed and the data for the toxicity of this substance may be found in Table XI. Even with doses of 25 mg. per 100 gm. of rat all animals were made very sick. There is, however, a sharp division between the fatal and the nonfatal dose and this varies some-

TABLE IX

PREPARATION B(W). RESIDUE FROM THE DIALYZED HCl + NaCl PRECIPITATE
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 2, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	135	50	3:53		1
2	100	100	3:54		1
3	90	150	3:55		1
4	115	200	3:56	Found dead 4/5/22 A. M.	

No apparent symptoms. Autopsy showed profuse peritoneal adhesions.

TABLE X

PREPARATION B(W"). THE PORTION OF B(W) SOLUBLE IN ABSOLUTE ALCOHOL
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 2, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	RECOVERED
	gm.	mg.	P. M.	
1	110	50	3:59	1
2	80	100	3:59	1
3	115	150	4:00	1
4	95	200	4:00	1

No apparent symptoms.

TABLE XI

VAUGHAN'S CRUDE SOLUBLE POISON FROM COAGULATED EGG WHITE
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

MARCH 8, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	265	25	2:52		1
2	265	25	2:53		1
3	265	50	2:54		1
4	230	50	2:55		1
5	140	100	2:55	3:10	
6	305	150	2:56	3:01	
7	345	125	2:57	3:01	
8	270	125	2:57	3:53	
9	305	150	2:59	3:04	
10	355	150	3:50		1

Animals very rapidly went into convulsions with emprosthotonos. There was no apparent respiratory difficulty. Nose, ears and legs did not become cyanotic and the animals died after extreme convulsive attacks. Heart beat long after respiration ceased. At 4:00 P. M. all others were still very sick and did not move. Autopsy showed nothing.

where between 50 and 100 mg. the latter being invariably and very rapidly fatal. Apparently the physical and chemical change involved in the coagulation of egg white does not prevent, although it may modify, the degree of toxicity of the toxic end product.

Preparation D(A).—Presumably nontoxic residue. This substance is nearly as toxic as the end product (Table XII) judged from the lethal viewpoint, except that death is more delayed.

Preparation D(B).—The precipitate which collected in the filtrate after alcoholic solution had cooled. From the data (Table XIII) it is quite apparent

TABLE XII

PREPARATION D(A). PRESUMABLY NONTOXIC RESIDUE
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	A. M.	
1	280	50	10:08		1
2	170	100	10:09	11:37	
3	225	150	10:10	11:40	
4	265	150	10:10	11:40	

Following injections animals screamed and seemed in pain. At 10:45 all animals were quiet, stupefied and inactive.

Autopsy showed intestines filled with blood. Abdominal walls were hemorrhagic.

TABLE XIII

PREPARATION D(B). PRECIPITATE WHICH COLLECTED IN FILTRATE AFTER ALCOHOL SOLUTION COOLED
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	A. M.	
1	300	50	10:01	Found dead 8:00 P. M.
2*	285	100	10:02	Found dead 2:45 P. M.
3	200	150	10:04	2:45 P. M.
4	230	130	10:05	11:42 A. M.

Main symptom was weakness with cyanosis.

Autopsy showed hemorrhage in the intestines up to large gut. Liver, lungs and heart normal.

*Found pregnant. Fetuses dead. Placenta very easily detached. Hemorrhage into the intestines and cecum very great.

that this substance possesses greater toxicity than the more soluble portion of Vaughan's Crude Soluble Poison from which it is separated. Although this preparation killed in smaller doses than Preparation D, death was delayed for a much longer period.

Preparation—Residue 2''.—This preparation was made by evaporating, on the water-bath, to dryness in acid solution the HCl precipitate obtained on neutralization of the alkaline alcohol solution. The dried residue was extracted with absolute alcohol and the alcoholic filtrate evaporated to dryness. Toxicity experiments (Table XIV) show a comparatively high grade toxicity being

more poisonous from the standpoint of deaths than the presumably Vaughan's Crude Soluble Poison of this preparation except that death was much less rapid.

Preparation—HCl Precipitate Dialyzed—Residue.—This preparation was toxic in comparatively large doses, see Table XV.

Preparation D(X).—Formed by extraction with absolute alcohol of the water insoluble residue of the Vaughan nontoxic portion D(A).

Even in doses of 50 mg. this substance is fatal (see Table XVI) although death takes place only after a number of hours.

From the experience gained with this series of preparations the query

TABLE XIV

PREPARATION RESIDUE 2". ABSOLUTE ALCOHOL EXTRACT OF HCl PRECIPITATE (ACID SOLUTION)

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	A. M.	
1	200	50	10:15	2:45 P. M.
2	120	100	10:15	11:15 A. M.
3	225	150	10:16	11:15
4	305	135	10:16	11:05

Animals were all in pain but this seemed to last only for a short time then animals became very weak and cyanotic. At 10:45 all animals were very inactive. Died with a slight convulsion.

Autopsy revealed intestines filled with fluid blood. Abdominal walls were hemorrhagic. Liver appeared necrotic. Fat had a peculiar glistening appearance. Lungs contained few hemorrhagic points.

TABLE XV

HCl PRECIPITATE DIALYZED—RESIDUE

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

APRIL 4, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	A. M.		
1	160	50	10:29		1
2	170	100	10:29		1
3	210	150	10:30	Found dead 8:00 P. M.	
4	145	200	10:30	Found dead 2:45 P. M.	

Autopsy showed nothing abnormal.

TABLE XVI

PREPARATION D(X). ALCOHOL EXTRACT OF WATER INSOLUBLE PORTION OF NONTOXIC RESIDUE

TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

April 4, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	A. M.	
1	80	50	10:45	Found dead 8:00 P. M.
2	215	100	10:45	Found dead 2:45 P. M.
4	170	150	10:45	Found dead 2:45 P. M.

Autopsy showed nothing abnormal.

arises whether the toxicity exhibited by all of these products is due to the same substance or to different groups. It is of course quite possible that in this series, for unknown reasons, fractionation into toxic and nontoxic portions was not so sharp. Indeed this would appear to be the simplest explanation although only by further study can this point be determined.

TABLE XVII

PREPARATION F. ORIGINAL NONTOXIC RESIDUE OF CASEIN
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 11, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.		
1	205	50	6:35		1
2	170	100	6:38		1
3	205	200	6:36	Found dead 9:00 P. M.	
4	205	250	6:41	Found dead 3/12/22 12:30	
5	160	300	6:42		1
6	190	350	6:45		1

Animals at first quite sick but at 9:00 P. M. seemed well except for a little weakness. Autopsy of No. 3 showed peritoneal hemorrhage and whole intestines filled with blood from ruptured vessel.

Preparation F.—Original nontoxic residue formed from casein by treatment according to Vaughan. This preparation possessed a low grade toxicity only, since in doses of 200 mg. and above, death occurred only after several hours (Table XVII).

Preparation F(1B).—With a view of testing the possibility of the formation of a toxic product by further treatment of the above (Preparation F) nontoxic residue, this substance was subjected to the action of the alkaline alcohol again, first for a period of six hours; the remaining residue for three hours and the final residue for a like period. Practically all of the material was finally rendered soluble in the alkaline alcohol solution. The united extracts were then carried through in the manner usual in the preparation of Vaughan's Crude Soluble Poison. The final preparation was called Preparation F(1B).

Comparing the toxicity of this substance with that of Preparation F it will be observed (Table XVIII) that the further treatment of the nontoxic residue has very greatly increased its toxicity, doses of 50 mg. per 100 gm. of rat proving rapidly fatal, death occurring sooner with increasing doses. It is therefore quite possible to form from the nontoxic residue a substance possessing poisonous properties to a degree equal to the crude soluble poison obtained from the untreated native protein. Such an observation lends support to the view that in the preparation of Vaughan's Crude Soluble Poison the protein molecule is not split into an inherent poisonous and a non-poisonous group but rather that toxic products may be formed by the treatment so long as any protein remains. In other words the treatment results in the formation of toxic substances presumably by hydrolytic change, perhaps similar in man-

ner to the formation of proteoses, from which indeed the crude soluble poison may be formed.

Preparation F(1A).—From the above treatment of the nontoxic residue there resulted the formation of a small quantity of material which would correspond to the nontoxic residue of Vaughan. Toxicity experiments (Table

TABLE XVIII

PREPARATION F(1B). FORMED BY FURTHER TREATMENT FROM THE NONTOXIC RESIDUE
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 8, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	A. M.	A. M.	
1	280	25	11:25		1
2	290	50	11:26	11:51	
3	290	75	11:27	11:35	
4	290	100	11:28	11:35	
5	290	125	11:29	11:34	
6	265	150	11:30	11:34	

Animals died in a similar manner to those injected with Preparation D. Toxic end product, i.e., convulsions with spastic movements and no real cyanosis or gasps even though the heart beat after respiration had ceased.

TABLE XIX

PREPARATION F(1A). CORRESPONDING TO RESIDUE OF VAUGHAN
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 17, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED
	gm.	mg.	P. M.	P. M.
1	135	50	2:46	Found dead 3/18/22 11:00 A. M.
2	175	100	2:47	4:50
3	180	150	2:48	4:45
4	200	200	2:50	5:02
5	170	250	2:52	4:06
7	140	350	2:55	3:55

Immediately after the injection the animals became furious and curled up about the point of injection as though in great pain. But after a short interval they seemed better. The last animal injected seemed to have rigid spastic paralysis of both hind legs from which it soon recovered. Animals died after a few slight convulsions and asphyxia.

Autopsy showed congested and injected intestines and in some places loops that were black and contained blood. This must have been produced by the injection, for lower down there was normal, formed feces. Lungs and heart were negative.

TABLE XX

VAUGHAN'S CRUDE SOLUBLE POISON FROM CASEIN
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 17, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	225	25	3:25		1
2	220	50	3:25		1
3	205	75	3:26		1
4	185	100	3:26	Found dead 3/18/22 11:00 A.M.	
5	240	125	3:27		1

No symptoms present.

XIX) showed a relatively high grade toxicity, although death was distinctly delayed.

Preparation G.—Vaughan's Crude Soluble Poison from casein prepared by the treatment outlined for Preparation A except that in Preparation G the alkaline alcohol solution was filtered while hot. This substance was not very poisonous since one rat died with a dosage of 100 mg. per 100 gm. of rat on the day following the injection, whereas an animal receiving 125 mg. recovered (See Table XX).

Preparation G(No. 1).—Material which separated on cooling from the alcohol solution possessed a high degree of toxicity (see Table XXI) as did Preparation G.

TABLE XXI

PREPARATION G(No. 1). SUBSTANCE INSOLUBLE IN ALCOHOL ON COOLING
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 17, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	180	50	4:08		1
2	200	100	4:09	Found dead 3/18/22 11:00 A.M.	
3	165	150	4:10	Found dead 3/18/22 11:00 A.M.	
4	135	200	4:10	4:42 P.M.	
5	150	250	4:10	5:40 P.M.	

Animals reacted like those of Preparation F(1A) with reaction to point of injection. Autopsy findings similar to Preparation F(1A).

TABLE XXII

NONTOXIC RESIDUE OF VAUGHAN
TOXICITY EXPERIMENTS—INTRAPERITONEAL INJECTION

March 27, 1922.

RAT	WT.	DOSE PER 100 GM.	INJECTED	DIED	RECOVERED
	gm.	mg.	P. M.	P. M.	
1	245	25	3:45		1
2	250	50	3:47	Found dead 3/28/ in A.M.	
3	305	75	3:47	6:40	
4	200	100	3:49	6:40	
5	255	150	3:50	6:40	
6	135	200	3:50	5:00	

Symptoms and autopsy findings as in G(No. 1).

Preparation G(No. 2).—Presumably nontoxic residue of Vaughan. Toxicity experiments show this preparation to possess distinctly poisonous properties since doses of 50 mg. or above proved fatal although death occurred with distinct slowness. On the other hand it possessed toxicity of a grade equal to or greater than the corresponding crude soluble poison obtained in the same preparation (see Table XXII).

From these latter preparations it must be quite apparent that a distinct separation of a toxic from a nontoxic portion by treatment with alkaline alcohol is not always effected.

SUMMARY

The preparation known as Vaughan's Crude Soluble Poison possesses for the rat a degree of toxicity similar to that for the guinea pig. The former animal may therefore be employed as experimental subject and so far as toxicity is concerned the interpretations derived from the one are directly applicable to the other animal.

The toxicity of various preparations of Vaughan's Crude Soluble Poison vary in degree whether made under similar or different experimental conditions. The division of the substance resulting from the treatment into toxic and nontoxic fractions is not uniformly sharp, since the so-called nontoxic residue may possess distinctly poisonous properties. Modifying the period of heating radically alters the toxicity of the products obtained, the general tendency being for a decrease in the toxicity of that portion known as Crude Soluble Poison and an increase in the toxicity of the so-called Nontoxic Residue. The whole matter may perhaps be explained on the basis of a change in solubility whereby under altered conditions certain substances are included in one or another fraction.

Subjecting coagulated egg white to the treatment employed in the formation of Vaughan's Crude Soluble Poison results in obtaining a preparation of high grade toxicity. It is noteworthy that all the various by-products fractioned in the formation of this substance gave evidence of toxic properties. This applies to the so-called nontoxic residue which was nearly as poisonous as the toxic end product. Apparently the change induced by the process of coagulation of the egg white facilitated rather than diminished the formation of toxic substances.

Vaughan's Crude Soluble Poison may be formed from the so-called nontoxic residue by further treatment.

The observations recorded above lead to the view that the substance or substances included in the term "Vaughan's Crude Soluble Poison" are products formed presumably by progressive hydrolytic change rather than that the protein molecule is split into a toxic portion and a nontoxic residue. On such an hypothesis may be readily explained the varying toxicity of different samples of Vaughan's Crude Soluble Poison, and the observation that by altering environmental conditions changes in toxicity of various fractions may be obtained.

It is therefore quite probable that Vaughan's Crude Soluble Poison cannot be regarded as a chemical entity but must rather be looked upon as a mixture of substances varying according to the conditions leading to its formation.

ORGANIC, PROTEIN AND COLLOIDAL SILVER COMPOUNDS; THEIR ANTISEPTIC EFFICIENCY AND SILVER-ION CONTENT AS A BASIS FOR THEIR CLASSIFICATION*

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INTRODUCTION

THE practical value of the high antiseptic efficiency of silver nitrate is limited by its side-actions: irritation and pain, astringency and corrosion. These may be largely avoided by the use of colloidal silver compounds which combine in many instances a fair degree of antiseptic action with a much smaller degree or entire absence of the irritant side-actions. The irritant and antiseptic actions of silver nitrate are due essentially to the free silver ions. The antiseptic action of the colloidal preparations has also been attributed to the presence and liberation of low concentrations of silver-ions, the concentration being so low as to be practically nonirritant, but still sufficient to be more or less antiseptic. Dreser[1] for instance, showed that the antiseptic action of the commercial "colloidal metallic silver" is destroyed if it is treated by agents that reduce silver ions to metallic silver (zinc dust, hydroquinon, pyrogallol); or by agents that bind silver ions (sodium thiosulphate, potassium cyanide, or sodium chloride). Gros[2] concludes that the colloidal silver preparations, notwithstanding their low concentration of silver ions, may be more efficiently antiseptic, in the presence of sodium chloride, than is silver nitrate, because the silver chloride from the colloidal silver forms a finer precipitate and therefore redissolves more readily than when it is precipitated from silver nitrate (except in very dilute solutions). This, however, could come into play only under certain conditions, notably with prolonged contact.

The numerous commercial brands of colloidal silver compounds may be grouped under a limited number of types.[3] Clinical experience has shown that these types differ in irritation and in antiseptic efficiency, so that their therapeutic field is not quite identical; but no exact comparisons have been made, for want of suitable quantitative methods. Even the classification of individual products, which should reflect the therapeutic grouping, was sometimes doubtful. Experiments on the restraint of bacterial growth have been made under various conditions by many observers; but the results have been contradictory, probably because of technical difficulties, so that they have but little value as a guide either to therapeutics or to classification. An encouraging prospect was opened by the observation of Dreser[1]

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that the growth of ordinary yeast furnishes an accurate and convenient index of silver ions, of antiseptic efficiency, and of irritation. Dreser applied the method to "colloidal metallic silver" preparations, and suggested that it might be useful for quantitative comparisons of organic silver compounds with silver nitrate; but he did not elaborate it further. Our experience with this method, somewhat modified as described in the appendix, shows that it furnishes reliable criteria for the comparative antiseptic efficiency of silver compounds, inorganic, protein and colloidal. The technic is simple and the data are quantitative, objective and of adequate uniformity. By determining the antiseptic efficiency in watery mixtures and in normal saline solution data are obtained which have a direct bearing on the various types of clinical application of silver salts; and which, moreover, indicate the relative proportion of ionized (irritant and astringent) silver; nonionized (nonirritant) but antiseptic silver, and inactive or reserve silver. We shall discuss this more fully later.

PRODUCTS EXAMINED

Silver nitrate was taken as the fixed standard and control. With this were compared fourteen organic or colloidal compounds, including the prominent commercial brands. Two or more specimens of each preparation (with one exception) were used, to determine the variability of the brands, making a total of 34 samples. Some were purchased recently, in unopened bottles; others were older specimens, on hand either in this or in the American Medical Association laboratory. These furnished the opportunity to compare the older and new preparations.

ANTISEPTIC EFFICIENCY IN AQUEOUS SOLUTIONS

This represents the *total* activity of ionized and nonionized silver. The measure of the activity is the "*inhibiting dose*," namely, the quantity of the compound in milligrams per 10 c.c. of mixture that is just sufficient to prevent gas formation within an hour, under the conditions of the experiment. This may also be stated as the "*inhibiting concentration*," 1 mg. per 10 c.c. representing a concentration of 1:10,000, etc. Silver nitrate was used for a comparison and as a check of correct technic since it gives reasonably uniform results: 0.25 mg. (1:40,000) nearly always prevented gas formation and was accepted as the standard; 0.22 to 0.20 may or may not be completely effective; 0.17 mg. always permits a slight formation of gas. Exceptionally a specially active yeast is found, which requires a higher concentration than 0.25 mg. In that case, a corresponding correction is made for the product with which it is to be compared.

The percentage variation of different tests of the other compounds was generally also small. It was not attempted to reduce it to less than 25 per cent from their mean, since this range is very much smaller than the difference between the various groups of compounds; the inhibiting dose ranging from 0.25 mg. (1:40,000) for silver nitrate, to 150 mg. (1:67) for one specimen of silvol. The data for the individual products and specimens are shown in columns 2 and 4 of Table I.

Different specimens of a given brand show satisfactory agreement, usually within 25 per cent, at most 50 per cent, of the smaller figure, for all the commercial products except those of the "Protargin Mild" (Argyrol) type, i.e., Group V of the Table. For these the strongest samples are two or three times as active as the weakest. In five of the six brands the recently purchased sample was the weaker. We cannot say whether this is more than a coincidence.

The Table shows that silver compounds arrange themselves, according to their total activity, into five distinct groups, separated by relatively wide

TABLE I

INHIBITING DOSES AND INHIBITING CONCENTRATIONS OF THE SPECIMENS

1. PRODUCT	2. WATER	3. NORMAL SALINE (0.8% NaCl)	4. WATER	5. NORMAL SALINE (0.8% NaCl)
Group I.	mg. per 10 c.c.	mg. per 10 c.c.	*1:*	*1:*
Silver nitrate	0.25	20	40000	500
Group II.				
"Silver nucleinate" Roche.	1.0	300	10000	30
Sophol. x	0.85	125	11500	80
Sophol. y	1.0	100	10000	100
Albargin 1922 (Silver gelatose)	1.2	—	8333	—
Mean of Group II	1.0	125	10000	70
Group III.				
Protargol (1)	2.2	110	4500	90
Protargol w	2.0	60	5000	165
Protargol 4x	2	125	5000	80
Protargol 5x	2.5	120	4000	85
Proganol /	2.5	100	4000	100
Proganol y	3.0	65	3300	155
Protargentum x	2.0	35	5000	280
Protargentum 1	2.0	70	5000	140
Protargentum A	3.0	50	3300	200
Silver proteinate	2.0	150	5000	65
Mean of Group III	2.0	95	4500	105
Group IV.				
Collargol x	10.	85	1000	115
Collargol /	11.	100	910	100
Mean of Group IV	10.5	93	955	108
Group V.				
Vargol X	11.	35	910	280
Vargol y	20.	45	500	220
Solargentum (1)	15.	40	670	250
Solargentum 2x	20.	60	500	165
Solargentum x	55.	80	180	125
Argyn (1)	20.	100	500	100
Argyn x	30.	75	330	130
Argyn y	55.	60	180	165
Cargentos (new)	35.	100	280	100
Cargentos (new process)	60.	—	167	—
Argyrol (1)	40.	500	250	20
Argyrol y	70.	40	145	250
Silvol x	60.	150	165	65
Silvol (1)	75.	100	135	100
Silvol (2x)	125.	175	80	55
Silvol of Current Sample	150.	—	67	—
Mean of Group V	38.	78	305	128

gaps. These are recapitulated in Table II. They correspond very satisfactorily to the generally accepted clinical types and clear up the position of some of the doubtful products. The "total activity" test is therefore an adequate and very convenient objective criterion for the grouping of the silver products.

The data have a further practical value in that they reflect the relative therapeutic antiseptic efficiency or the relative concentration of the solutions that must be used to secure equal antisepsis within an hour, provided

TABLE II

THE GROUPING OF SILVER COMPOUNDS ACCORDING TO THEIR ACTIVITY IN AQUEOUS SOLUTIONS

GROUP	COMPOUNDS	INHIBITING QUANTITY IN MG.	INHIBITING CONCENTRATION
I. Inorganic salts.	Silver nitrate	0.25	1:40,000
II. Protargin fortissimum.	Silver nucleinate Sophol Albargin	0.85-1.2	1:13,300 to 1:8,333
III. Protargol.	Protargol Proganol Protargentum Silver proteinate	2-3 (mean 2.2)	1:5,000 to 1:3,300
IV. Collargol.	Collargol	10-11	1:1,000 to 1:900
V.* Argyrol.	Vargol Solargentum Argyn Cargentos Argyrol Silvol	11-150 (mean 38)	1:900 to 1:67

*Group 5 may be thought to contain members with too wide a range; it is, however, but little greater than the difference in different lots of the same product.

that the surfaces are fairly clean, or when the solutions are used by lavage. The prophylactic efficiency against gonorrheal infection comes under this heading, for there the action must be exerted within a few minutes after application to secure the maximum protection.

To facilitate the use of the data for therapeutic purposes, Table III has been prepared, showing the concentration of the various groups that is equivalent in antisepsis to a 1:1000 solution of silver nitrate. The activity in a saline medium is also shown for convenience and will be discussed in the next section.

TABLE III

CONCENTRATIONS OF SILVER COMPOUNDS EQUAL IN ANTISEPTIC VALUE TO SILVER NITRATE 1:1000

GROUP	DISSOLVED IN WATER	IN PRESENCE OF 0.8 PER CENT NaCl.
I. Silver nitrate	1:1000	1:1000
II. Protargin fortissimum	1:250	1:140
III. Protargol type	1:115	1:210
IV. Collargol	1:25	1:216
V. Argyrol type	1:7.6	1:256

ANTISEPTIC EFFICIENCY IN SALINE SOLUTION

The presence of sodium chloride decreases very greatly the antiseptic efficiency of silver nitrate; to a smaller extent that of the organic silver compounds. This, of course, is due to the precipitation of the silver as chloride, and the decrease is proportional to the silver-ion content of the aqueous solution. However, silver chloride is not absolutely insoluble; a small quantity escapes precipitation or is redissolved, and continues to act as an antiseptic. This solution is favored by the presence of colloids, and for this reason the colloidal silver compounds, with their protective proteins, may be more effective than silver nitrate, as pointed out by Gros. However, this occurs probably only under special conditions, namely when the quantity of active silver is so small that hours or days are required to check bacterial growth. This would be valuable in clinical conditions that permit practically continuous application; and it would then have the material advantage that the nonionized silver is nonirritant. To this we shall recur later.

The inhibiting dose and concentration of our silver compounds in the presence of sodium chloride is shown in columns 3 and 5 of Table I. A mere glance at column 5 shows that the activity of all the groups, except silver nitrate, has been reduced to a nearly uniform level. The inhibiting concentration ranges between 1:280 and 1:20, without any reference to the groups. The mean is just 1:100; and half of all the specimens lie between 1:80 and 1:125.

This means that the interference of the chloride is proportional to the activity of the preparation in watery solution, which, again, merely means that reduction of activity is due to the inactivation of silver ions. What remains is the activity of the silver chloride, and it is interesting and of practical importance to note that nearly the same amount of this is dissolved for equal weights of all the compounds, so that the nature of the compound itself is practically negligible. The only notable exception is silver nitrate, which, contrary to expectations, retained an activity of 5 times the average figure. Presumably the preponderance of silver ions is so great, with this salt, that some remained free, in accordance with the law of mass action.

RATIO OF IONIC AND NONIONIC ACTIVE SILVER

Table III shows that, if applied in normal saline for an hour, silver nitrate 1:1000 is equivalent to the colloidal protein-silver preparations in the concentration of about 1:250. Clinically, however, silver nitrate is not applied in saline solution, but in water; and before it meets with enough chloride to disionize it, it has caused so much irritation that it cannot be used frequently. The protein compounds, on the other hand, cause so little irritation that they can be applied continuously in much stronger solution. Clinically, therefore, the comparison is more likely to be, not with ¼ per cent, but with 5 or 10 per cent protargin strong, or 10 to 40 per cent of protargin mild. For continuous application these would be superior.

The reason for this superiority, then, lies in a more favorable ratio, not of absolute efficiency, but of efficiency in proportion to irritation. This in turn rests on the ratio of nonionic but active silver, to ionic silver. The higher this

ratio, the more suitable is the compound for continuous application. The ratio is reflected by dividing the antiseptic efficiency in normal saline (representing the nonionic active silver) by the efficiency in water (representing active nonionic plus ionic silver); or conveniently, by using Table I, and dividing column 2 by column 3. The quotients for the mean data of the groups are shown in Table IV.

TABLE IV

QUOTIENT OF NONIONIC TO TOTAL SILVER, REPRESENTING ALSO THE RATIO OF ANTISEPTIC EFFICIENCY TO IRRITATION

Group I.	Silver nitrate	0.0125
Group II.	Protargin fortissimum	0.008
Group III.	Protargol type	0.0023
Group IV.	Collargol	0.1
Group V.	Argyrol type	0.5

The silver nitrate probably shows a more favorable figure than it really deserves, for the reasons explained above (imperfect precipitation of silver ions); but the clinical superiority of the argyrol type over the protargol and other types, where irritation has to be avoided, is faithfully reflected. The protargol type can be rendered as nonirritant as the argyrol type by being dissolved in saline solution; but this of course involves a proportional loss of antiseptic activity, to about $\frac{1}{50}$th of the activity in water. In other words, solution in normal saline converts the protargol type of silver into the argyrol type of silver. It does not, of course, change the protein, and therefore does not fully reproduce the demulcent actions of argyrol.

INACTIVE OR RESERVE SILVER

If one calculates the quantity of silver nitrate that would produce the antiseptic effect of one gram of the colloidal preparation and subtracts the weight of silver in this quantity of silver nitrate from the total weight of silver in one gram of the colloidal preparation, the remainder represents essentially silver that does not participate in the antiseptic action (in one hour under standard conditions), and which is therefore *inactive silver*. We are not prepared to say whether this is entirely useless, or whether it may be slowly converted into active silver, and thus play a part in very prolonged contact with the tissues, either directly (analogous to the experiments of Gros), or by replacing part of the active silver that becomes inactive by absorption or by conversion into a permanently insoluble form, etc. This would be more properly termed "reserve-silver." This phase of the subject needs further investigation.

RESIDUAL BALLAST OR VEHICLE

This includes the total nonsilver part of the compound, i.e., what remains when the silver-content is subtracted from the total weight. It includes the oxygen of the silver oxide, the protein of the silver proteinate, and the protein present as stabilizer or as diluent. The protein has considerable practical importance; as a demulcent in local application; as a dangerous provocative of anaphylactoid reactions in patients with specific protein-hypersusceptibility

or on intravenous injection. Such instances have been reported; but the subject lies too far outside of the scope of the present investigation to justify further discussion.

PERCENTAGE OF THE VARIOUS FORMS OF SILVER

This was calculated for each specimen by the methods explained in the appendix, but only the median figures for the five groups need be reproduced. They are shown in Table V.

TABLE V

MEDIAN PERCENTAGE OF THE VARIOUS FORMS OF SILVER

	IONIC SILVER	NONIONIC ACTIVE SILVER	TOTAL ACTIVE SILVER	INERT SILVER	TOTAL SILVER	BALLAST
Group I. Silver nitrate type	62.7	0.8	63.5	0.	63.5	36.5
Group II. Protargin fortissimum	16.7	0.07	16.8	3.2	20.	80.
Group III. Protargol type	7.05	0.21	7.26	1.04	8.3	91.7
Group IV. Collargol type	1.34	0.18	1.52	76.5	78.	22.
Group V. Argyrol type	0.32	0.2	0.55	20.5	21.	79.

This Table illustrates the progressive decrease of ionic silver and consequently of irritation and activity from the silver nitrate to the argyrol group; the uniformly small activity of the nonionic silver; consequently, the total activity practically parallel to the ionic silver; the independence of the inert silver from the other forms; and the variability of the ballast-content.

CONCLUSIONS

1. The antiseptic value of silver salts and organic or colloidal silver compounds may be determined by their inhibiting action on the yeast-fermentation of sugar.

2. Determinations with watery solutions permit the grouping of the commercial products into five types, shown in Table II, which correspond with the clinical groups.

They, therefore, furnish a satisfactory laboratory method for assigning new compounds to their proper groups.

They are also a useful check on the uniformity of different specimens of the products. The only brands that showed much variation belonged to the protargin mild (argyrol) type.

The determinations in water would be fairly parallel to the immediate or early antiseptic effects, especially on clean surfaces or with lavage.

3. The presence of sodium chloride reduces the activity of all the compounds to a low level, which is practically the same for all the compounds. The difference between the activity in water and the activity in saline corresponds to the content of free silver ions and is a measure of the irritant and astringent effects.

The activity in saline solution is probably more nearly representative of the clinical conditions of prolonged contact.

The tables show the data for individual specimens and groups. The methods and further details are explained in the appendix.

Appendix

In this have been included such details as would not have much interest for the general medical reader, but which serve to establish the validity of the method, or which deserve preservation for other reasons.

1. Technic of the determinations

The materials needed are a constant temperature water-bath, ordinary test tubes (1.5 x 15 cm.) and smaller test tubes (0.8 x 10 cm.); a 10 per cent solution of cane sugar; fresh cakes of compressed yeast (Fleischmann's); solutions of the drugs to be examined (10-20 per cent); pipettes; chloride-free water. Make a 4 per cent yeast sugar mixture by triturating a weighed quantity of yeast in the 10 per cent sugar solution, place 10 c.c. portions of the mixture into a series of the large test tubes in racks; add the desired quantity of the drug in solution (preferably 1 c.c. or less) to the mixture; shake well; fill a small test tube with the mixture from the large test tube and at once invert it into the larger, being careful to allow no air bubbles to rise into the small tube; this is readily accomplished by rapidly inverting the small tube and dropping it into the solution in the large tube, although occasionally the presence of air bubbles necessitates refilling the small tube; place the tubes in the rack into the water-bath at 38° C. In one hour measure the amount of gas formed in the small test tube in centimeters. The reading should be done within one or two minutes after taking the tubes from the bath, to obviate contraction of the gas by cooling.

A control test should always be made with silver nitrate, 15 mg. and 25 mg. per 10 c.c. If 15 mg. should give us gas; or should 25 mg. give more than a trace of gas, the inhibitory dose of silver nitrate for that yeast must then be determined, and the reading for the compound corrected by the formula: True inhibiting dose of compound: determined inhibiting dose of compound : : 25: determined inhibiting dose of silver nitrate. For example a certain sample of yeast was inhibited by 250 mg. of silvol and by 40 of silver nitrate. The true inhibiting concentration of silvol was therefore

$$\frac{X}{250} = \frac{25}{40} \; ; X = \frac{25 \times 250}{40}$$

The experiments in sodium chloride solution are made in a similar manner by substituting 0.8 per cent sodium chloride for water.

A 4 per cent yeast suspension in 10 per cent cane sugar solution was used, as these percentages gave a suitable amount of gas formation in one hour; with a lower yeast concentration (2 per cent) the fermentation was delayed and made the experiments extend over an unnecessarily long time (some two hours) to give a similar gas formation. Fresh yeast suspensions were used for each experiment as on standing at room temperature for an hour or less sufficient gas was formed in the mixture to vitiate results.

Preliminary experiments were made with each sample of drug using a wide range of dosage such as 1, 2, 4, 8, 16, 32 mg., etc; and then the more accurate dosage was determined, with these results as a basis. The readings were made in centimeters of gas formed and not in the actual quantity by

volume, as the experiments were comparative, and therefore, the relative amount of gas formed under the action of the different drugs was desired and not the actual amount.

A. *Accuracy of the Method.*—Control experiments, i.e., with yeast and sugar only, were made in all cases and from a series of 61 of these it was seen that the natural experimental variations were practically negligible, with the average CO_2 formation of about 7 cm., with the extremes of 4.3 to 9 cm. of CO_2; 24 of these were within 7 to 8 cm., 13 within 6 to 7 cm. and the remainder somewhat below or above these quantities.

B. *Effect of NaCl on Control* growth without drugs; NaCl up to 3 per cent has no appreciable effect on CO_2 formation. No experiments were made with higher concentrations.

C. *Presence of NaCl in Materials Used.*—As traces of sodium chloride might interfere by precipitating some of the silver, chloride-free water was always used; the yeast sugar mixture contained mere traces of chloride in the filtrates and it will be seen later that such traces have no appreciable effect on the silver action.

D. *Effect of Dilution on CO_2 Formation.*—The addition of 1 c.c. of water (10 per cent) has no effect; 1.5 c.c. may cause a slight decrease in CO_2 formation, still within normal variations, but above that there is a readily demonstrable decrease. However, when it was necessary to add the drug in 1 c.c. or greater quantities, the drug was first put into the test tube, diluted to 5 c.c. and 5 c.c. of a double strength sugar-yeast mixture added (20 per cent sugar solution, 8 per cent yeast); this gave the usual 10 per cent sugar and 4 per cent yeast mixture and avoided any possible effect of dilution.

2. DETERMINATION OF THE SODIUM CHLORIDE CONCENTRATION THAT NEUTRALIZES THE INHIBITORY QUANTITY OF THE SILVER PREPARATIONS

In the saline solution (0.8 per cent) which was used in this work the sodium chloride was in large excess over the silver content of any of the compounds examined, including $AgNO_3$, so that it seemed worth while to determine the concentration of sodium chloride that would just neutralize the silver in the inhibiting quantity. The question was approached in two ways; first mixing the chloride and silver preparations and allowing them to stand for 10 minutes to 2 hours before adding the yeast sugar mixture; secondly, by adding the silver preparation to the yeast sugar mixture made up in saline solution of the desired strength. The first method was tried as it was thought that part of the silver might act on the yeast before combining with the sodium chloride. In either case a very large excess of sodium chloride was necessary to have an appreciable effect on the action of silver nitrate and protargol, which were taken as examples of the silver compounds. This is shown in Table VI. When silver compounds and chloride solutions were previously mixed somewhat smaller quantities of sodium chloride neutralized the silver, but a large excess of the chloride was still necessary.

The results indicate that under the experimental conditions the compounds formed by silver and sodium chloride may be actively antiseptic: a question which we have stated is not the province of this paper to discuss.

TABLE VI

DECREASE OF ANTISEPTIC ACTIVITY ACCORDING TO EXCESS OF SODIUM CHLORIDE

EFFECT OF SODIUM CHLORIDE ON THE SILVER COMPOUND ACTIVITY	IONIC SILVER ATOMS : CHLORIDE ATOMS AS 1 :	
	SILVER NITRATE	PROTARGOL
Negative or slight decrease	25	15
Moderate decrease	40	30
Large decrease	100	60+
Abolition of activity	200	200–300

3. CALCULATION OF THE TYPES OF SILVER

(a) *Total Silver.*—The percentage stated by manufacturers was accepted, since this has been repeatedly verified by independent workers.

(b) *Active Silver.*—The inhibiting dose of silver nitrate contains 0.159 mg. of silver. This was taken as the unit of "active silver," i.e., the quantity of the compound that just inhibited yeast was considered to contain 0.16 mg. of active silver. For instance, the inhibiting dose of a sample of protargol was 2.0 mg.; the percentage of active silver in the sample would therefore be

$$\frac{100 \times 0.16}{2} = 8.0 \text{ per cent.}$$

(c) *Inactive or Reserve Silver.*—This is obtained by subtracting the active silver (b) from the total silver (a). In the above example, this is 8.3 – 8.0 = 0.3 per cent.

(d) *Ionic Silver.*—This corresponds to the quantity of "active" silver that is destroyed by sodium chloride. In the protargol sample quoted the inhibiting dose in water was 2 mg.; in normal saline it was 125 mg. Therefore, the chloride destroyed the activity of $\frac{123}{125}$ of the active silver. Since the total active silver was 8.0 per cent, the ionic silver amounts to $8 \times \frac{123}{125} = 7.8$ per cent.

(e) *Nonionic but Active Silver.*—This corresponds to the difference between the total active and the ionic silver; in our example, this is 8.0 – 7.8 = 0.2 per cent.

(f) *Ballast or Vehicle.*—This includes all components except silver, and is obtained by subtracting the percentage of total silver from 100; in the example, 100 – 8.3 = 91.7 per cent.

4. OLD SOLUTIONS

Solutions of these preparations may preserve their activity fairly well as is shown by the fact that a 25 per cent solution of solargentum that was in the laboratory for about four years lost but a third of its activity (the inhibiting dose increasing from 15 mg. of a fresh solution of the same preparation, to 20 mg. in case of the old solution). On the other hand, they may deteriorate considerably for a 5 per cent solution of protargentum lost much of its activity in the same time; the inhibiting dose increased from about 2 to 8 mg.

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NOTES ON D'HERELLE'S PHENOMENON. ADAPTATION OF BACTERIOPHAGE ANTAGONISTIC TO BACILLUS DYSENTERIAE AND OTHER BACILLI TO VARIOUS COCCI. DEVELOPMENT OF A POLYVALENT BACTERIOLYSANT*

(Preliminary Report)

By Earl B. McKinley, A.B., M.D., Ann Arbor, Mich.

According to D'Herelle, perhaps the first demonstration of the phenomenon of "bacteriophagy" was made by Hankin, who in 1896 reported the bactericidal action of the water of the Ganges and Jumna rivers in India for the cholera vibrio. With others it has usually been assumed that Twort was the first to describe this phenomenon since Hankin was unable to destroy the bactericidal power of the water by heating to 115° C. for a half hour in an autoclave, a temperature which destroys bacteriophages. It is not impossible, however, that the water from the rivers Hankin examined contained bacteriophages as the phenomenon has been demonstrated by Dumas in filtrates of water from the Seine. In recent years the literature dealing with this very interesting phenomenon has increased steadily. Investigators in this field have dealt with many different phases of the subject, including sources of bacteriolysants; variation in titer; effects of chemicals, temperature and concentration on the degree of lysis; nonspecificity of the filterable "substance"; separation of cultures into resistant and sensitive types by action of bacteriophages; inoculations in animals, therapeutic action, etc.

In 1915 Twort noted in a culture of staphylococci, transparent areas in which no cocci grew. Touching one of these transparent areas with a sterile platinum loop and then drawing the loop across the surface of a 24-hour agar culture of staphylococci, he found after a few hours, a streak marking the track of the loop which had become clear and transparent. Filtering this material from these transparent areas through a Berkefeld, he found that the filtrate would dissolve and kill most of the organisms in fresh staphylococcus agar cultures even in dilutions of one to a million. This work was confirmed recently by Gratia and he called attention to the fact that this is the only gram-positive organism for which an observation of transmissible autolysis has been made.

Bruynoghe and J. Maisin have also worked with staphylococci and state in one of their reports that the staphylococcus bacteriophage could not be made to have any effect upon either the typhoid group of B. dysenteriae. These investigators have worked with bacteriolysants which have been obtained from cultures of staphylococci while the following experiments are

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based upon the adaptation of bacteriolysants antagonistic to B. dysenteriae and other bacilli to various cocci. D'Herelle stated that there is only one species of bacteriophage and this can acquire activity against any organism. He found that a bacteriophage in addition to its lytic power against the organism causing the patient's infection could acquire a lytic activity against B. proteus, B. coli, B. typhi murium, B. typhosus, B. paratyphosus A and B. B. diphtheriae, B. gallinarum, B. subtilis, V. cholerae, staphylococci and other organisms.

The material used in the following experiments was originally obtained from D'Herelle, a bacteriolysant antagonistic to shiga dysentery. Two c.c. of our stock bacteriolysant known as "31 A" was used in a 1:10 dilution. Stock 31 A on titration was active in about a 1:6,000,000,000 dilution against shiga dysentery.

I. FIRST SERIES. SET A

To 1 c.c. of the 1:10 dilution of stock 31 A were added 49 c.c. of glucose blood broth. The media was prepared by adding glucose broth to whole blood which was allowed to stand for several hours in the cold room and the supernatant fluid taken off. The P_H concentration was 8.2.

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. diluted bacteriolysant inhibiting shiga dysentery	1	2	3	4	5	6
5 c.c. bacteriolysant as controls	9	10				
5 c.c. Glucose blood broth	7	8				

To Tubes 1 and 7 were added three drops of an eight hour blood broth culture of Meningococcus. Three drop serial transfers were then made from Tube 1 through Tube 6. Tube 8 was kept as a broth control and Tubes 9 and 10 as bacteriolysant or lytic principal (LP) controls.

The entire series was then incubated at 38 degrees.

Results after incubation for 24 hours:—Tubes 1 to 6 inclusive distinctly cloudy.
Tube 7 cloudy.
Tubes 8, 9 and 10 clear.

48 hours:—Tubes 1 to 7 markedly cloudy.
Tubes 8, 9 and 10 clear.

Entire series of 10 tubes were filtered through a Berkefeld after 60 hours.

Second series:

The filtrate from the first series was then incubated 24 hours. It remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP*	1	2	3	4	5
5 c.c. GBB*	6	7	8	9	10
5 c.c. GBB	11				
10 and 15 c.c. LP	12	13			

*LP—Lytic Principal.
GBB—Glucose Blood Broth.

To Tubes 1 and 6 were added three drops of an eight hour glucose blood broth culture of Meningococcus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 11 served as a broth control and Tubes 12 and 13 as lytic principal controls. The series was then incubated at 38 degrees.

Results after incubation for 24 hours: Tubes 1 to 5 inclusive were cloudy but not so much as Tubes 6 to 10.
Tubes 6 to 10 very cloudy.
Tubes 11, 12 and 13 remained clear.

36 hours: Practically the same except the degree of cloudiness was more marked though the relative turbidity remained between the LP tubes and broth tubes.
Control tubes remained clear.

At the end of 48 hours the entire series was filtered through a Berkefeld and 10 c.c. of fresh GBB of same p_H concentration were added to the filtrate.

Third series:

The filtrate from the second series was incubated 24 hours and remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP	1	2	3	4	5
5 c.c. GBB	6	7	8	9	10
5 c.c. GBB	11				
10, 10 and 15 c.c. LP	12	13	14		

To Tubes 1 and 6 were added three drops of an eight hour blood broth culture of Meningococcus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 11 GBB control. Tubes 12, 13 and 14 LP controls.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 inclusive almost entirely clear.
Tubes 6 to 10 cloudy.
Control tubes clear.

24 hours: Tubes 1 to 5 slightly cloudy.
Tubes 6 to 10 very cloudy.
Control tubes clear.

36 hours: Tubes 1 to 5 remained the same.
Some debris in bottom of tubes.
Tubes 6 to 10 markedly cloudy.
Control tubes remained clear.

At the end of 48 hours all tubes were filtered through a Berkefeld and 10 c.c. fresh GBB of p_H 8. 2 concentration were added to the filtrate.

Fourth series:

The filtrate from the third series was incubated for 24 hours. It remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP	1	2	3	4	5
5 c.c. GBB	6	7	8	9	10
5 c.c. GBB	11				
15 c.c. LP	12	13	14		

To Tubes 1 and 6 were added three drops of an eight hour blood broth culture of Meningococcus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 11 GBB control. Tubes 12, 13 and 14 LP controls.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 remained clear.
Tubes 6 to 10 were cloudy.
Control tubes clear.

24 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 cloudy.
Control tubes clear.

36 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 very cloudy.
Control tubes clear.

48 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 cloudy (markedly).
Control tubes clear.

72 hours: Readings the same.

At the end of 72 hours the series was filtered through a Berkefeld. During the above experiments of Set A, Set B had been built up in precisely the same manner. The remaining 1 c.c. of the 1:10 dilution of stock 31A was diluted with 49 c.c. of GBB of p_H 8.2 and this material was carried through

the fourth series with meningococcus the same as Set A, excepting 20 c.c. was carried along with shiga dysentery. The results with Set B were exactly the same as with Set A and complete inhibition of growth of the meningococcus did not occur until the fourth series was reached.

At this point filtrates of Set A and B were combined. The combined filtrates were then carried through the 5th, 6th, 7th, 8th, 9th and 10th series with meningococcus with complete inhibition of growth in the LP tubes attending each series. When cultures to which a few drops of the adapted lytic principle had been added were plated out, there were found many transparent areas in which no cocci grew and if one of these transparent areas was touched with a sterile platinum loop and then drawn across the surface of a 12 and 24 hour blood agar culture of meningococcus, a clear streak developed in about eight hours. A titration at this point, with the already extensively diluted Stock 31 A, showed inhibition in dilutions of 1:500,000 to 1:800,000 with six, eight and ten hour cultures of the organism. Forty c.c. fresh glucose blood broth were added to the combined filtrates.

II. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH BROTH CULTURES OF STAPHYLOCOCCUS ALBUS

The combined filtrates of Set A and B were incubated for 48 hours and remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5
5 c.c. GBB	6	7	8	9	10
5 c.c. LP	11				
5 c.c. GBB	12				

To tubes 1 and 6 were added three drops of a six hour culture (GBB) of Staphylococcus albus.

Three drop serial transfers were then made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 12 served as GBB control while Tube 11 served as LP control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear. Tubes 6 to 10 cloudy. Control tubes clear.
24 hours: The same except Tubes 6 to 10 were more cloudy.
36 hours: The same except Tubes 6 to 10 were more cloudy.
48 hours: The same. Tubes 6 to 10 markedly cloudy.
60 hours: The same. LP tubes clear.
72 hours: LP tubes remained clear.

III. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF STAPHYLOCOCCUS AUREUS

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	12
5 c.c. GBB	6	7	8	9	10	11

To Tubes 1 and 6 were added three drops of a six hour GBB culture of Staphylococcus aureus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10.

Tube 12 LP control. Tube 11 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear. Tubes 6 to 10 cloudy. Control tubes clear.

24 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 very cloudy.
Control tubes clear.
36 hours: Reading the same except Tubes 6 to 10 were more cloudy.
72 hours: Tubes 1 to 5 remained clear.
Tubes 6 to 10 markedly cloudy.
Control tubes clear.

IV. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF STAPHYLOCOCCUS CITREUS

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a six hour GBB culture of Staphylococcus citreus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 remained clear.
Tubes 6 to 10 cloudy.
Control tubes clear.
24 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 very cloudy.
Control tubes clear.
48 hours: Same except Tubes 6 to 10 more cloudy.
72 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 markedly cloudy.
Control tubes clear.

V. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF MICROCOCCUS TETRAGENOUS

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a six hour GBB culture of Micrococcus tetragenous.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 cloudy.
Control tubes clear.
48 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 very cloudy.
Control tubes clear.
72 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 markedly cloudy.
Control tubes clear.

VI. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF PNEUMOCOCCI. GROUPS 1, 2 AND 3. INASMUCH AS THE RESULTS WERE THE SAME FOR THE DIFFERENT TYPES THE RESULTS ARE GIVEN UNDER ONE GENERAL HEADING

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To tubes 1 and 6 were added three drops of an eight hour GBB culture of pneumococci.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 almost clear.
Control tubes clear.
24 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 definite cloud.
Control tubes clear.
48 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 very cloudy.
Control tubes clear.
72 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 markedly cloudy.
Control tubes clear.

VII. REACTION OF BACTERIOLYSANT ADAPTED TO MENINGOCOCCUS WITH GBB CULTURES OF HEMOLYTIC STREPTOCOCCI

This strain was exceedingly virulent and was obtained from a fatal case of infection in the University Hospital. The first results obtained with this organism using serum broth media were not dependable due to the appearance of a heavy precipitate in the inoculated tubes presumed to be globulin.

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP adapted to Meningococcus	1	2	3	4	5	11
5 c.c. GBB	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a ten hour GBB culture of hemolytic streptococci.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 GBB control.

Results after incubation at 38 degrees for 12 hours: All tubes clear.
24 hours: All tubes except 6 and 7 were clear. 6 and 7 slightly cloudy.
48 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 cloudy.
Control tubes clear.
72 hours: Tubes 1 to 5 clear.
Tubes 6 to 10 markedly cloudy.
Control tubes clear.

VIII. The question arose as to whether the results obtained in the preceding experiments could be obtained direct with the bacteriolysant which was being carried along on shiga dysentery, that part of the original dilution of the 1:10 dilution of stock 31A saved from our Set B. These 20 c.c. had been carried along with shiga dysentery through several series and new broth had been added from time to time and complete inhibition was being obtained with the organism. Consequently this bacteriolysant was tried with the organisms used in the above experiments. The results were uniformly the same, showing absolutely no inhibition of growth of the organism during the first and second series. For example we give below the results obtained with staphylococcus aureus.

The filtrate was incubated for 24 hours and remained clear.

	TUBE	TUBE	TUBE	TUBE	TUBE	TUBE
5 c.c. LP inhibiting shiga dysentery	1	2	3	4	5	11
5 c.c. Glucose broth	6	7	8	9	10	12

To Tubes 1 and 6 were added three drops of a six hour broth culture of Staphylococcus aureus.

Three drop serial transfers were made from Tube 1 through Tube 5 and from Tube 6 through Tube 10. Tube 11 LP control. Tube 12 broth control.

Results after incubation at 38 degrees for 12 hours: Tubes 1 to 5 cloudy.
Tubes 6 to 10 cloudy.
Control tubes clear.
24 hours: All cloudy except control tubes.
48 hours: All cloudy except control tubes.
No inhibition in any of the LP tubes.

Identical results were obtained as above with micrococcus tetragenous, staphylococcus citreus, pneumococci and hemolytic streptococcus. There appeared to be a slight amount of inhibition in the first series with staphylococcus albus and there was complete inhibition in the second series.

CONCLUSIONS

1. It is possible to bring about adaptation of a bacteriolysant antagonistic to shiga dysentery to the meningococcus and other cocci.

2. This adaptation might be termed a "group" adaptation since by adapting the bacteriolysant to one coccus it was found also to have been adapted to other cocci.

3. These experiments suggest that there is only one species of bacteriophage and this can acquire activity against any organism.

4. A staphylococcus bacteriophage or a coccus bacteriophage developed by adaptation can be readapted to its original organism, as in this case shiga dysentery.

5. The possibility of a polyvalent bacteriophage adapted to a large number of organisms and inhibiting the growth of all at the same time.

6. The reaction of organisms to Gram stain is apparently not a factor in "bacteriophagy" as shown by adaptation to the pneumococci, micrococcus tetragenous, streptococcus and staphylococci of the gram-positive group and the meningococcus of the gram-negative group of organisms.

THE BIOLOGICAL ASSAY OF PITUITARY EXTRACT*

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I. INTRODUCTION

In the case of certain widely used drugs, notably the digitalis group, cannabis, suprarenal extract and pituitary extract, exact methods of chemical assay are not at the present time available. For these drugs the so-called "biological assay" methods are in use. It is recognized of course that quantitative chemical methods are the goal to be sought, but lacking these, it is necessary to have in the meanwhile some way of determining the relative strengths, so that the preparations which are made available to the physician may be uniform. For most of the drugs mentioned above there have been devised more or less satisfactory methods, as shown by the fact that the preliminary reports of the various committees working on the question of revision of methods for the next edition of the United States Pharmacopeia show little inclination to change other than minor details of the present methods. The assay process of pituitary extract, Liquor Hypophysis of the U.S.P. IX, has however been subjected to a number of criticisms on many scores.

The necessity for some agreement as to method of assay is clearly shown by the facts indicated in Table I, which gives in comparative form the strength of ten of the preparations available to the physicians of the United States. Of these, five purport to be of the strength indicated in the U.S.P. IX, and practically all of them carry the statement "physiologically standardized." If the use of physiological assay methods and a Pharmacopeial standard results in the manufacture and marketing of preparations differing as widely in strength as these do, then clearly something is wrong. As a matter of fact the terms as thus used mean one thing to the manufacturer and to the physician another. To the maker they are a statement that the successive lots of the extract which he puts on the market from time to time are uniform in strength. In justice to the manufacturers it should be said that in the writer's experience this has always been found to be the case. To the physician on the other hand there is at least the inference that a given preparation is uniform in strength with any other preparation for which similar claims are made. He is usually quite surprised to discover that this is not actually so. This difference in strengths is a potential and an actual source of danger to his patients. Suppose for example that an obstetrician

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The investigation herein reported was initiated in 1920 at the suggestion of the Chairman of the Sub-Committee on Biological Assays of the Revision Committee of the United States Pharmacopeia. After the work was well along toward completion it was learned that Dr. H. H. Dale of the Medical Research Council of the British Medical Association was carrying out a similar study. His report which is very complete and thorough was most generously made available in the press proof.

has been in the habit of using the preparation given as Number 9 in this table, and for some reason he is unable to get this preparation with which he is perfectly familiar and the dose of which he has worked out by experience. He uses instead Number 2, which so far as the literature attached indicates, is exactly the same, being "physiologically standardized" and of the U.S.P. strength. Actually it is at least three times the strength of the preparation to which he has become accustomed. The possibilities of accident are sufficiently obvious.

After some thought the conclusion has been reached that it would be unwise to give the names of the preparations whose strength is given above. The interpretation of the facts is sufficiently clear, namely that a method of assay and suitable standards should be adopted so as to insure the marketing of extracts which are of a uniform strength. If the names of the preparations are given, the first conclusion to which a casual reader might come is that the stronger preparations are the better ones. This is by no means the case. In the first place, the correct dosages of pituitary extract have not

TABLE I

GIVING THE RESULTS OF ASSAYS OF TEN COMMERCIAL PREPARATIONS OF PITUITARY EXTRACT IN TERMS OF ONE OF THEM, NUMBER 1.

NUMBER OF PREPARATION	RESULTS OF ASSAY	
	OXYTOCIC METHOD	PRESSOR METHOD
1.	10	10
2. U. S. P.	–	10 plus
3.	7–8	10 plus
4.	6	8–9
5.	6	6
6. U. S. P.	5–6	5–7
7.	–	5
8. U. S. P.	3	4–5
9. U. S. P.	3	3–5
10. U. S. P.	1–	2

been worked out, in the very nature of the case cannot be worked out, until there is a greater agreement among the preparations, but there are in the literature a large number of cases of untoward events resulting from the administration of too large amounts of the extract, or of its improper use.[1] It is the teaching of a number of the obstetricians that the doses used should be reduced. The manufacturers themselves have recognized this, and in some cases have reduced the strength of their preparations, or have put on the market solutions of lower concentration, marked for obstetrical use. But even under these circumstances accidents have happened. Under such conditions the best preparation for any physician is not the strongest, but the one with which he is most familiar. It makes no difference to him whether the preparation he uses is of a strength to rank in the first place or the fifth place in such a table, so long as it is strong enough to accomplish what he desires, and is uniform from sample to sample. His protection at present is to know one preparation and to stick to it. This is not the intent of the Pharmacopeia. In the case of other potent drugs, the official preparations conform to a definite strength, regardless of the manufacturer. It should be thus with

Liquor Hypophysis. Of course it does not lie in the province of the pharmacology laboratory or of the United States Pharmacopeia to say what dose the clinician shall use but just as obviously is it necessary that some procedure shall be adopted which will insure that the physician shall be able to buy pituitary extract anywhere on the open market and be assured that the preparation obtained corresponds in activity to those to which he has been accustomed.

In the second place, some of these extracts purport to be 20 per cent extracts, in terms of the fresh moist gland, some are 10 per cent, and in some cases the strength is not stated. The theoretical strength, when given, does not always correspond to that actually found in laboratory examinations. From the manufacturer's viewpoint, a 10 per cent extract which ranks fifth is a better preparation than a 20 per cent preparation which ranks seventh. From his viewpoint that preparation which contains the maximum strength per unit of fresh gland material is the best, being the most economical. The clinician however is concerned with this point only insofar as it affects the cost to him of the finished product. If the extract is potent, the efficiency of the manufacturing process concerns him not at all.

The fundamental principle of the physiological assay is that it measures the amount of the substance examined required to bring about the same quantitative changes in the behavior of some physiological mechanism as is produced by a known amount of a standard substance having the same pharmacological action. It has generally been insisted that the assay method should be based upon the activity for which the drug is used in therapeutics. However, as Dale[2] has clearly pointed out, it is only necessary that the method measure in some way the activity of the same substance as that having the action for which the drug is used in its therapeutic applications. For example, in the case of the assay of pituitary extract, if it can be clearly shown that the constituent which produces the rise of blood pressure when injected into the veins of a laboratory animal is the same as that which causes contraction of the uterus in the pregnant woman, then it is perfectly permissible to use the laboratory determination of the pressor activity as an index of the oxytocic strength of the drug as used in the clinic. This point will be taken up again later. "Useful Drugs"[3] gives the following summary of the action of the extract of the posterior lobe of the pituitary gland, or the Liquor Hypophysis of the U.S.P. IX: "It stimulates the uterus to contract, whether intact or excised, it increases the blood pressure by peripheral constriction, stimulates the kidney to increased secretion of urine, causes broncho-constriction, stimulates the smooth muscle of the mammary gland * * * and in certain pathological conditions at least stimulates the intestine and bladder to increased contraction." Of necessity one must take the measure of the physiological activity along one of these lines as an index of its therapeutic strength.

II. THE PRESSOR METHOD OF ASSAY

The assays of Table I are made by two methods, the pressor method, that of comparing the doses of known and unknown extracts required to

produce equal changes in the blood pressure, and the oxytocic method, which consists in determining the doses of known and unknown having the same activity on the excised uterus of the virgin guinea pig. These two methods are the only ones which have received any serious attention in the standardization of pituitary extract.

The pressor action of the extract of the pituitary body was described by Oliver and Schäfer.[4] Howell[5] showed the action was confined to extracts of the posterior lobes. Hamilton[6] suggested the use of the pressor reaction in determining the strength of extracts. Roth[7] compared a number of commercial preparations by this method and in 1916 Hamilton and Rowe[8] reported in detail the method as applied to the standardization of one of the commercial extracts. The method has been used repeatedly in this laboratory, essentially as outlined by Hamilton and Rowe. Dogs are used, and chloretone dissolved in olive oil is given intraperitoneally. The vagi are cut to eliminate reflexes, and the blood pressure is taken from the carotid in the usual way. Alternate doses of standard and unknown are given at fifteen-minute intervals until a dose of the preparation being assayed is found giving the same pressure increases as the standard. The strengths of the two extracts are then in inverse proportion to the doses used. The results of assays carried out by this method are in general more or less parallel to those obtained by the isolated uterus method (Table I). The technic, while not difficult, requires more attention than the excised uterus method, so that it actually seems less tedious (Dale, 1922, with which the writer agrees).

There is one very good reason why such a test should not be adopted as an official method of assay for pituitary extract. It is quite certain that the most important use of pituitary extract is for its action on the uterus. If the pressor and oxytocic bodies were the same, it would be quite proper, as pointed out in a previous paragraph, to use the pressor action in the dog as a measure of the oxytocic action in the human species. But Dudley[9] has shown that it is possible to separate in part at least the oxytocic and pressor bodies, and in a later paper not yet published he gives evidence that there are probably *two* pressor substances as well as the substance which stimulates the uterus.[10] Under such circumstances it is quite illogical to use the pressor test to determine the oxytocic activity. In this connection it has been reported several times that the values for particular extracts as found by the two methods do not always run parallel.[2, 7, 11]

III. THE OXYTOCIC METHOD OF ASSAY

The oxytocic action of extracts of the hypophysis was first pointed out by Dale in 1906.[12] He later gave a clear picture of the action and localized the point of action as being on smooth muscle.[13] Kehrer recommended the use of the isolated uterus as a test organ for determining the strength of ergot preparations.[14] Engeland and Kutscher used the isolated uterus of the cat as a means of determining the pharmacological action of the various fractions which they separated from pituitary extract.[15] It was Dale and Laidlaw,[16] however, who first definitely worked out a method for comparing quantitatively the strengths of pituitary extracts using the isolated uterus

of the guinea pig. They were led to the use of this organ by certain theoretical considerations. A method which is to give quantitative results must not be complicated by the question of tolerance. To put it differently, the amplitude of response of any physiologic mechanism to given doses of a drug must not be conditioned by the preceding dose. When pituitary extract is injected into the blood stream, there is an increase of blood pressure which is maintained over a relatively long period. The pressor substance is eliminated or destroyed rather slowly, so that the circulation returns to its normal condition quite gradually, and a certain degree of tolerance to succeeding doses remains. "It seemed quite probable that a thin strip of isolated muscle, suspended in a large volume of Ringer's solution, which could be rapidly removed and replaced by a fresh volume, would have a much better chance of recovering its original condition by the rapid washing out of the principle, or the reduction of its concentration below the threshold of activity." They found the isolated horn of the uterus of the young virgin guinea pig most satisfactory, as when suspended it assumes a condition of low tonus, broken only by occasional small contractions. As a standard they used a 10 or 20 per cent extract of fresh moist gland, sterilized in ampoules and kept in this form. It was clearly pointed out in the conclusion of their paper that the use of such a standard made the method essentially a comparative one, not giving absolute values. An absolute standard was not suggested because of the lack of a substance having an action qualitatively similar to that of pituitary extract. Roth[17] and Heidelberg, Pittenger, and Vanderkleed[18] verified the value of the method as developed by Dale and Laidlaw. Roth recommended the use of histamine as a standard against which to compare the unknown extracts, in place of the "standard extract" of previous workers. In 1915 the ninth revision of the United States Pharmacopeia appeared, incorporating Roth's suggestion and outlining the method which is official at the present time. The U.S.P. IX gives the following requirement for the Liquor Hypophysis: "One mil of Solution of Hypophysis, diluted 20,000 times, has the same activity on the isolated uterus of the virgin guinea pig as a 1 to 20,000,000 solution of beta-iminazolylethylamine hydrochloride when tested as directed by the United States Hygienic Laboratory." (As a matter of history it may be stated that this action was not taken by the Sub-Committee on Biological Assay, which explains why the method was not included in the section on "Biological Assay.")

The directions of the United States Hygienic Laboratory are those given by Roth.[7] Young virgin pigs weighing from 275 to 350 grams are recommended. A piece of uterus one to two centimeters long is used, and is weighted enough to overcome the tonus resulting from the necessary manipulation. A temperature of about 39° C. is used. Further details are given as to the arrangement of stock bottles, thermostat, etc., most of which details are modified in the various laboratories using the method.

Since this method was made official, a number of criticisms and suggestions have been made, chiefly on the part of the representatives of the various manufacturing houses, as they are the ones to use the test most extensively. In 1918 Spaeth[19] gave a very good summary of these objections, pointing

out that they fall into three classes: (1) objections as to the nature of the test; (2) objections to the use of histamine (beth-iminazolyl-ethylamine) as a standard; (3) objections to the requirement as to strength of the finished commercial extract as given in the U.S.P.

(1) *Objections to the Nature of the Test.*—Other methods of assay have been suggested, as pointed out above, based on other physiologic properties of the extract. Dale and Laidlaw compared extracts as to their pressor action, and at that time concluded that for practical purposes the results were parallel to those obtained by the oxytocic method, but held that the latter method was more accurate, and more desirable on theoretical grounds. At the present time Dr. Dale does not hold to this view, and reports that he has examined preparations in which the oxytocic and pressor activities were not parallel.[2] Hamilton[6] and Hamilton and Rowe[8] consider that the pressor method gives a more accurate picture of the therapeutic strength of the extract than does the oxytocic test. They point out that histamine, against which the U.S.P. requires that Liquor Hypophysis be tested, has no value in obstetrics, and ask whether it may not be merely a coincidence that pituitary extract acts to stimulate the excised uterus and the gravid uterus as well. As a matter of fact histamine acts on the uterus *in situ* but has other systemic actions which make its use in obstetrics impracticable. The fundamental objection to the use of the pressor method has been pointed out in an earlier paragraph.

Under the heading of objections to the nature of the test, Spaeth groups also the statements of technical difficulties involved. These have been met to a greater or less degree by all who have had occasion to use the method. The greatest difficulty is that of obtaining satisfactory uteri. Roth recommends those from virgin guinea pigs of a weight of from 275 to 350 grams. Uteri from animals which are or have recently been pregnant, or are in heat, are discarded. The ideal uterus is one which when suspended in the bath of Ringer's soon reaches a condition of minimum tonus upon which are superposed a succession of contractions of mild intensity. When the proper amount of pituitary extract is added to the bath the tonus increases fairly rapidly and quite regularly. (Fig. 1.) The amplitude of tonus increase is in a general way proportional to the concentration of the extract. If the concentrations are kept within certain limits, the contractions are submaximal, and when the pituitary extract is washed off there is a prompt return to the original low level. However, some uteri are found which do not give this satisfactory picture. Some are hyperirritable, giving maximal contractions to all effective concentrations. Others give such large spontaneous contractions that it is difficult or impossible to differentiate between the contraction due to the drug action and that due to the spontaneous action. These difficulties may at times be avoided by increasing the tension against which the uterus has to contract, but again, this procedure may and often does result in a decrease in the irritability of the strip, making it useless for the test. This means a loss of one or two hours in experimenting with an unsatisfactory strip. Another difficulty, not so frequent of occurrence, but more serious, is the obtaining of strips which will not give to two successive applications of the same concentration of pituitary extract, the same ampli-

tude of contraction. Pittenger[20] states that this condition can often be remedied by increasing the weights exerting tension on the uterine strip.

These difficulties have been encountered by every worker, and are mentioned in practically every paper dealing with the use of guinea pig uteri for assay purposes. Various individuals have attempted to get around the trouble by trying to learn some method of selecting suitable animals before sacrificing them. Practically all workers are agreed that the larger animals, weighing above 300 or at most 350 grams, and those pregnant or in heat, should be rejected. Trendelenberg and Borgmann[21] have studied this question in some detail, and insist that only those uteri which are obtained from small pigs, and which give very small spontaneous contractions, should be used. They maintain that with the uteri from larger pigs the amplitude of the contraction does not of necessity follow parallel to the concentration of pituitary extract, but that often increasing the concentration acts to shorten the latent period rather than to increase the amplitude of contraction. They do not use animals weighing over 250 grams.

The most recent statement on the matter is in the paper by Burn and Dale.[2] They find that if young females are kept out of sight and smell of the males, from the time they are weaned, and are used when weighing from 200 to 300 grams, and moreover are not used during heat, that practically all of these difficulties may be avoided. The experience of the writer confirms this, as does that of Eckler (personal communication).

Of the above writers, all of whom admit to a greater or less degree the difficulties to be met in applying the oxytocic test, only Hamilton and Rowe state that it should not be retained as official.

Kochmann[22] has recently described a method of getting around the difficulties offered by hyperirritable uteri, and at the same time he attempts to make possible the utilization of the larger sizes of guinea pigs which may be available. He reduces the irritability of the uteri by reducing the calcium content of the Ringer-Locke, and if necessary by adding magnesium chloride to the solution, before treating the uterus with pituitary extract. Histamine is used as a standard. The sequence of application of successive doses is entirely different from that ordinarily used, and he utterly ignores the changes in irritability which occur spontaneously in such preparations. The method has not been tried in this laboratory.

Spaeth[23] realizing the difficulty of the uterine method, suggested a more objective method, i.e., that of comparing the extracts to be standardized by measuring the degrees of contraction which they produced in the melanophores of the isolated scales of a fish. The method, so far as known, has not been tried by other pharmacologists, who seem for some reason to feel that it represents an excursion too far into the fields of the zoologist. It is of course open to the objection pointed out by Dale for the pressor method of assay, in that it is not proved that the substance which causes the melanophores to contract is the same as that which causes the uterus to contract.

(2) *Objections to the Substance Used as a Standard.*—There have been many objections to the use of histamine as a standard substance. These have been well summarized by Spaeth.[19] The objections can be placed in two main

groups; (*a*) those concerned with the uniformity of the various specimens of the salt and the stability of the salt or its solution; (*b*) those concerned with the qualitative similarity or dissimilarity to pituitary extract with respect to its action on the excised uterus.

(*a*) Roth[17] investigated the physiologic activity of the three samples of histamine available to him before recommending its use as a standard, and found them to be uniform. He also found that solutions did not lose in activity when autoclaved in ampoules on three successive days. Pittenger and Vanderkleed[24] however reported that the dry salt did not keep. They prepared solutions which were set aside for a year. At the end of that time fresh solutions of the same theoretical strength were prepared from the same stock of crystals. The year old solutions were stronger than those made up freshly. This they interpret as meaning a deterioration of the dry salt. They do not mention the possibility of the salt being deliquescent. If this were the case, of course the new solutions would be weaker than the old. Spaeth[19] agrees with the conclusions of these authors. The samples of histamine available to him did not agree either in their physiologic strength or their physical constants. He pointed out that the salt is hygroscopic, which introduces the possibility of a very considerable error in weighing. These considerations precluded its use as a standard and he recommended as a substitute the use of potassium chloride.

It had previously been shown by this author that the melanophores of fish scales responded in a quantitative manner to potassium chloride solutions and inasmuch as he believes the melanophores to be smooth muscle cells much modified; he argued that the smooth muscle of the uterus ought to behave in a similar manner. Experimental work seemed to bear out his argument and in 1917 he recommended the substitution of potassium chloride as a standard substance, suggesting that in order to make this standard conform to U.S.P. requirements, a commercial preparation should have in a 1:4,000 dilution the same effect on the isolated uterus as a 0.14 per cent solution of potassium chloride in Ringer's solution.

(*b*) There are various hints in the literature that the action of pituitary extract and histamine are not exactly parallel, but no evidence given until the recent paper by Burn and Dale.[2] This of course would entirely preclude the use of histamine as a standard.

(3) *Objections to the Strength of the Present Requirement.*—Few if any of the preparations in use at the present time actually correspond in strength to the standard given in the U.S.P. IX. They are instead generally from three to ten times this strength. For example, the chemist of a manufacturing concern writes that in his laboratory it is the practice to consider an extract as satisfactory when in a dilution of 1:200,000 it has the same activity on the isolated uterus of the guinea pig as a 1:20,000 solution of histamine hydrochloride. In other words his preparation is just ten times the official strength. Whether this preparation is too strong is another question, but at any rate it is the view of the writer that the majority of the preparations now sold, and with which this generation of physicians is familiar, are far stronger than the Liquor Hypophysis of the present Phar-

macopeia, and that in formulating a new standard, the strength should be set so as to approximate as nearly as possible the average of accepted commercial practice, which after all gives at least an indication of the requirements of the medical practice. If the profession can be supplied with a uniform preparation, then it will be in a position to work out the proper doses for the various clinical indications.

IV. EXPERIMENTAL

(1) *Potassium Chloride as a Standard.*—It seemed worth while to investigate the possibility of the use of potassium chloride as a standard, since it seemed to meet clearly the requirements set forth by Spaeth as desirable in a standard, namely (1) qualitative similarity to pituitary extract with respect to its smooth muscle action; (2) parallelism in the changes in irritability of uterine strips to the extract and the standard substance (this was emphasized by Spaeth as being of most fundamental importance); (3) stability and ease of obtaining in pure form; (4) relatively high concentration required to affect the uterus. He pointed out in connection with this last point that the chance of error in dilution of solutions of a substance such as histamine is extremely large, since histamine is active in very dilute solutions. Since potassium chloride seemed to meet all of the above requirements, a number of standardizations were carried out with its use.

The technic followed was essentially that used in every laboratory where the activity of smooth muscle is studied. The apparatus consisted of a tube with a capacity of 100 c.c., in which the uterus was suspended. This tube was surrounded by several liters of water in a copper bath, kept at constant temperature by a small gas burner, regulated by a thermostat. The tube was arranged so that the waste fluids could be drawn off at the bottom, and fresh Ringer's added in the same way. This fresh solution was run through a large glass coil having a capacity of something over 100 c.c., which was placed in the water-bath. The solution from the stock bottle was siphoned through this coil, and was thus heated to the same temperature as the fluid in the tube surrounding the uterus. This arrangement makes it possible to have a constant supply of fresh Ringer's at the proper temperature and at the same time does not keep any more of the stock heated than is immediately necessary. Eckler has described an apparatus which is quite similar.[25] The required amounts of pituitary extract and standards, whether potassium chloride or histamine were blown from pipettes into the tube. The accuracy of dilution obtained by this method of addition and dilution in the tube is of course not so great as that described by Roth,[7] in which the proper dilution is made up outside the muscle chamber, and then used to replace the Ringer's in which the muscle has been suspended. However, it has been felt that the possible errors of dilution by the method as used in this laboratory have been well within the limits of accuracy of comparisons, and in making the dilutions in this manner, the stimulus of exposing the muscle to the air momentarily has been avoided. The solution in which the muscle is suspended is aerated from a tank of compressed oxygen.

The Ringer-Locke used in the earlier comparisons was of the following composition:

NaCl	0.9%
$CaCl_2$	0.026%
KCl	0.042%
$NaHCO_3$	0.01%

Later the composition was changed to agree with the formula given by Burn and Dale,[2] in which the bicarbonate is increased to 0.05 per cent, dextrose is added to make 0.05 per cent, and magnesium chloride 0.0005 per cent. This solution has proved more satisfactory, and is now used in all isolated uterus work. It should be emphasized that the salts used must be of the highest purity obtainable. The strength of the final dilution of the potassium chloride standard solution was that recommended by Spaeth, 0.14 per cent.

At first the results of the experiments with the use of this method

Fig. 1.—The lack of parallelism between the changes in irritability toward potassium chloride as compared with pituitary extract. KS = 0.14% KCl, the standard of Spaeth. At the beginning pituitary extract in 1:2500 dilution is equal to KS. A dilution of 1:3700 is too weak, but the second application of 1:3700 is too strong. There is an interval of 97 minutes between the two applications of 1:3700. At S and S′ the drum was stopped.

were quite promising, so much so that at the meeting of the Revision Committee in Philadelphia in 1920, a preliminary report was made in which the use of the KCl standard was recommended. Further experience however has shown that the substance is unsuitable. The results of a number of experiments are given here to show the basis for the objections to potassium chloride. Fig. 1 shows the curve from an experiment in which the changes in irritability to potassium chloride and pituitary extract are not parallel. At the beginning of the experiment a 1:2,500 dilution of pituitary extract is the equivalent of 0.14 per cent KCl, and a 1:3,700 dilution is clearly inferior to the KCl standard. After an interval of fifty minutes, a 1:3,700 dilution is just as clearly too strong. In other words the changes in irritability are not parallel. Either the irritability of the strip to potassium chloride is reduced, or else it is increased to pituitary extract. It

might be mentioned in passing that this particular preparation of pituitary extract was by far the weakest examined. It is Number 10 of Table I. In Fig. 2 are shown the results of a comparison of potassium chloride and pituitary extract on the two horns of a uterus. The first comparison (*a*) was carried out immediately on removal of the uterus, while (*b*) was made twenty-two hours later, the second horn having been kept in the meanwhile on ice. The pituitary extract used was diluted the first day and kept in the interval in the cold. In the tracing from (*a*) to 1:5,000 dilution of pituitary extract is about the concentration required to equal the standard. Clearly 1:6,000 is too weak, and 1:4,000 too strong. In the second part,

Fig. 2.—The different response of two horns of the same uterus to the potassium chloride standard. *A* was used immediately on removal from the body. *B* was kept on ice 22 hours. The same pituitary extract was used on both strips. In *a* the KCl standard is clearly stronger than pituitary extract, 1:6000 (6M), while in *b* it is just as clearly weaker than 1:12,500 (12.5M).

1:12,500 is clearly too strong. It was later found that at least at the end of the experiment 1:20,000 was about correct. Such an experiment merely exaggerates the differences in the changes in irritability toward the two substances, but it offers additional evidence that the two substances do not act in a qualitatively similar manner on the excised uterus. The changes differ in magnitude but not in kind from those shown in Fig. 1.

Another objection to the use of potassium chloride as a standard is that the strips subjected to its influence do not retain their activity so long as those subjected merely to the action of pituitary extract. This is a

conviction based on the impression obtained from a large number of standardizations in which potassium chloride was used, rather than on the results of experiments planned to test the point. It is of course of little importance as compared with the more serious objection of the lack of parallelism in the changes in irritability toward the two substances used. It is not intended to imply that the types of responses just discussed occurred in every instance. As a matter of fact the first difficulty with this method came from an entirely different source. The first dilutions of pituitary extract used were those given in the Hygienic Laboratory Bulletin No. 115,[19] which proved to be entirely too strong, and it took some time to discover that it was necessary to use very much more dilute solutions in order to avoid giving concentrations which produced maximal contractions. It is hard to understand the concentrations given by Spaeth. It has been necessary to use dilutions twenty to fifty times greater than his in order to avoid maximal contractions. The particular extract studied in the experiment from which Fig. 1 is taken was the only one which came anywhere near his recommendation, namely that a satisfactory extract should correspond to the 0.14 per cent potassium chloride standard when used in a 1:4,000 dilution.

It is felt that the considerations as given, especially the lack of parallelism in changes in irritability toward the two substances, entirely preclude the use of potassium chloride as a standard substance in the assay of pituitary extract.

(2) *Histamine as a Standard.*—The standard adopted by the U.S.P. IX for pituitary extract is of course histamine hydrochloride. As pointed out in the discussion of the literature, a large number of objections have been raised to the use of this substance, these being concerned with the stability of the salt and the qualitative similarity of its action on the uterus to that of pituitary extract. The impression among most of the pharmacologists seems to be that it is quite stable under ordinary conditions. For example Dudley[9] has shown that a solution of histamine phosphate is not affected by standing at room temperature (17° C.) for two hours with an equal amount of 2N sodium hydroxide. However, Pittenger and Vanderkleed[24] seemed to offer some proof of its deterioration, and Spaeth held the same view. Roth,[17] at the time of making the first recommendation as to its use, offered evidence that it did not lose in activity in the practical use of the substance in making a number of assays. At the meeting of the Society of Pharmacology and Experimental Therapeutics at New Haven in 1921, most of the pharmacologists there present seemed to question the lack of stability. Certain experiments have been carried out here which seem capable of interpretation only on the ground of instability.

The histamine used was the hydrochloride, "Imido" Roche, obtained from Hoffmann LaRoche Company, New York. On February 4, 1921, 50 mg. were weighed out and dissolved in 50 c.c. of freshly distilled water. One c.c. fractions of this solution were then placed in glass ampoules which were sealed and sterilized by boiling. These ampoules were kept in the dark, and were used as the stock from which fresh dilutions were made up as needed. In January, 1922, 20 mg. of the same specimen of histamine as used in 1921,

and dissolved in 20 c.c. of distilled water. This fresh solution was compared with the ampoules of a year earlier. The results are shown in Fig. 3. They clearly indicate that the new solution is distinctly weaker than the old. This result is capable of two explanations, either the crystals had taken up water, or they had lost in activity. The stock of crystals had been kept during this interval in a tightly stoppered glass bottle, which was not opened during that time.

The new solution was then placed in ampoules, and part of it sterilized by boiling on three successive days. It was then compared with the unboiled new solution. The results are given in Fig. 4. Here again, the boiled solution is weaker than the unsterilized solution. There seems to be no explanation except that there is a loss of activity in the boiling. The results have been checked to eliminate the possibility of errors of dilution.

Fig. 3.—Experiment to show the difference between a fresh solution of histamine, and a solution of the same stock which had been kept a year. Solution "O" was made up February 4, 1921, in 1:1000 strength, and sterilized by boiling in glass ampoules for three successive days. "N" was made up in the same strength January 23, 1922, and examined without sterilization. The new solution is clearly inferior to the old in strength.

When the stock of crystals was examined in September of 1922, it was clearly seen that they had taken up some water. Whether the loss of activity on boiling can be corroborated by other workers or not, the hygroscopic action of this particular salt of histamine should preclude its use as a standard.

The other group of objections to the use of histamine as a standard are those which have to do with the qualitative similarity in the action of histamine and pituitary extract. With the exception of the recent paper of Burn and Dale[2] there have been no definite statements on this point, other than those of Roth,[7] who finds that the actions of the two substances are qualitatively similar. The Physiological Assay Committee of the American Pharmaceutical Association suggested that this point be investigated, being evidently somewhat skeptical on this score. Dr. Dale, in a personal communication, has shown tracings which bring out very clearly the fact that in

some cases there is a lack of parallelism in the changes in irritability, similar to that in the case of potassium chloride. The writer has had similar experiences, and in addition has had experiments in which it was possible to get a fairly good approximation on one strip of uterus, which did not agree with subsequent comparisons made on the other half of the same uterus. (Fig. 5.) In the first part of this experiment the amounts of the two solutions given to cause the same amplitude of contraction stand in the ratio of one to six. In the second part of the same experiment, carried out on the other horn of the same uterus, the ratios are of one to eight, one to six being clearly too strong. It should be stated that this stock solution of histamine was diluted to one part in fifty thousand, so that the final concentration applied to the uterus in the first experiment would be 1:16,666,000.

It seems to the writer that the lack of parallelism in the changes in irritability as between histamine and pituitary extract, the hygroscopic na-

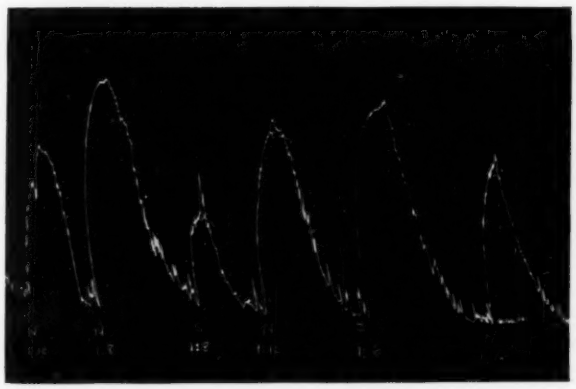

Fig. 4.—Experiment to show the loss of activity of histamine during the sterilization process. A solution of histamine was made up fresh, and placed in ampoules. Part of the ampoules were sterilized by boiling for twenty minutes on three successive days. The contents of one of these ampoules was then diluted and compared with a similar dilution of one of the same batch of ampoules which had not been subjected to the boiling process. *N*, not boiled; *S*, boiled. Dilutions in millions. Clearly *S* is weaker than *N*.

ture of the histamine, and the evidence as to instability under the conditions of use are enough to preclude the use of histamine as a standard.

V. DISCUSSION

It would seem that the lack of a substance having a qualitative similarity to pituitary extract in its action on the excised uterus, would make it necessary to use some preparation of the gland itself as a standard. This was of course the first standard employed, and in those laboratories in which the pressor method has been developed, it is still used. Others who actually have used histamine, have maintained that a pituitary preparation of some sort would be preferable. The first objection to such types of standards is of course that they do not make possible exact evaluations. This objection was pointed out by Dale and Laidlaw and later by Roth. It still constitutes a very serious objection, but in the absence of any better plan, it seems necessary to ignore it. After all, the purpose of an assay method is to insure the preparation of extracts of uniform potency for the medical profession. If a

pituitary preparation can be made which can be easily duplicated in any laboratory, without important variations in strength, then this preparation will meet the practical requirements of a standard, and also the theoretical one of qualitative similarity of action. The paper of Burn and Dale makes very definite recommendations as to the preparation of such a standard from the fresh pituitary glands. They have convinced themselves of the uniformity of the content of oxytocic substance from gland to gland, and are therefore using a preparation made from six or eight fresh glands by a rigidly pre-

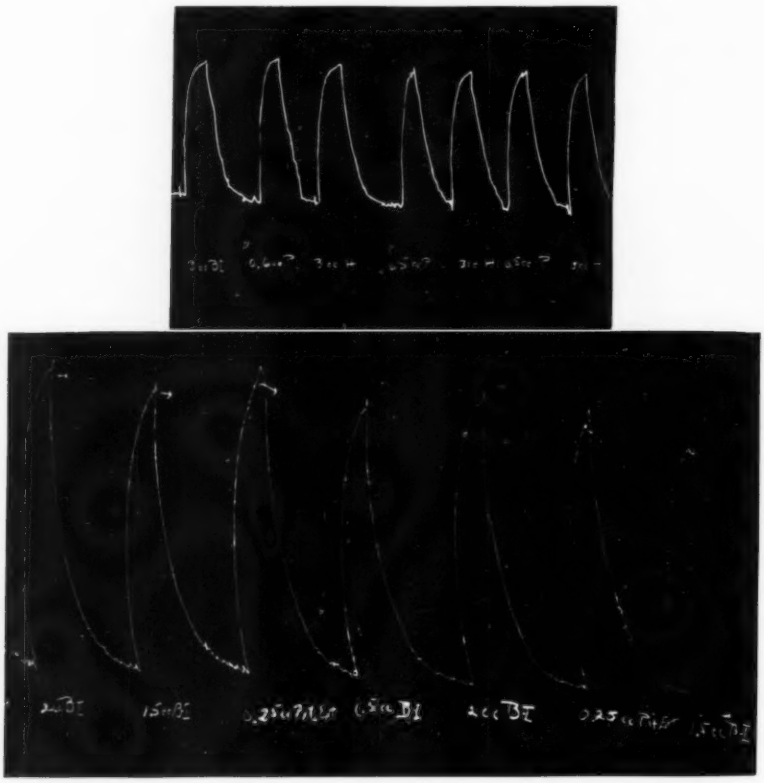

Fig. 5.—Comparison of histamine and a pituitary extract on the two horns of the same uterus. In *a* the amounts of the two substances added from the pipette stand in the ratio of one to six. In *b*, which was carried out about five hours after removal of the uterus from the pig, the ratio is one to eight, one to six being clearly too strong. The histamine solution was a 1:50,000, and the bath had a capacity of 100 c.c., so that 0.3 c.c. of histamine solution gives a concentration of 1:16,666,000.

scribed process. The data which they have presented seem convincing, but are being worked over in this laboratory with the cooperation of others concerned.

VI. SUMMARY

It has been shown that potassium chloride and histamine are not suitable substances for the standardization of pituitary extract. Since no other material which is yet available has an action qualitatively similar to that of pituitary extract, it is felt that a preparation of the gland itself should be used as a standard.

Because of the evidence given elsewhere as to the nonidentity of the oxytocic and pressor substances in the pituitary gland, the pressor method should not be employed in the assay of a drug which has its chief use for its oxytocic action.

It is expected that a definite recommendation will be made very shortly as to the details of preparation of a standard from the pituitary gland itself, together with the details of the exact method to be followed in the biological assay of pituitary extract.

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LABORATORY METHODS

A NEW METHOD FOR THE DETERMINATION OF CALCIUM, MAGNESIUM, POTASSIUM AND SODIUM IN HUMAN BLOOD*

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THE determination of calcium, magnesium, potassium and sodium has been carried out by several investigators. The usual method consists of getting rid of the protein, (either by oxidizing it with a mixture of concentrated sulphuric and nitric acids or by precipitating the protein with trichloracetic acid) and determining calcium as oxalate, magnesium as magnesium-ammonium-phosphate or magnesium-ammonium-arsenate, and the total amount of potassium and sodium as sulphates; potassium as potassium-cobalt-nitrate or potassium-chloroplatinate, and sodium from the difference between the total amount of the alkali sulphates and the calculated amount of potassium sulphate or directly as sodium pyroantimonate.

This method is open to several objections. It has been pointed out by no less an authority than the official Association of the Agricultural Chemists that the cobalt-nitrite method for the determination of potassium yields inaccurate figures on account of variations in the formula of potassium-cobalt-nitrite. This objection has been disregarded by several investigators, who must have reasoned that the named variations are of little significance in the determination of such small quantities of potassium salts as are usually found in the blood.

The authors are in a position to state that the variations may affect considerably the accuracy of the results. The proof was furnished by determining the amount of potassium in an aqueous solution which contained all four above-named metals in the same quantities as they are usually met with in the blood. Platinum is too expensive to be used in analytical work and for that reason the chloroplatinic acid has been superseded by other reagents (perchloric acid, sodium-cobalt-nitrite).

The formula of either magnesium-ammonium-phosphate or magnesium-ammonium-arsenate is also subject to variations and should not be used for the determination of magnesium, although the perspective of determining magnesium colorimetrically which it offers (liberating, aerating and Nesslerizing the ammonia of magnesium-ammonium-phosphate or magnesium-ammonium-arsenate) is rather fascinating.

The determination of the inorganic constituents of the blood is still in

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the macrochemical stage. We do not yet possess such methods for the determination of calcium, magnesium, etc., which would rival in delicacy those used for the determination of urea, creatinin, uric acid or sugar. The quantity of calcium and magnesium usually found in the blood being very small, we have our doubts as to the possibility of obtaining reliable results with less than 25 c.c. of blood. This amount should be considered the minimum, and if larger amounts are obtainable, the better.

We must not forget that little is yet known as to the rôle of such metals as calcium and magnesium in the human body, and therefore, the more reliable the results of analysis, the better the cause of the knowledge is served. We have plenty of hypotheses with the main weight resting on speculations and fanciful assumptions instead of reliable analytical data. Before the amount of the blood necessary for the determination of the inorganic constituents can be reduced, colorimetric methods should be devised which would equal in their accuracy the familiar blood chemistry methods.

The oxidation of blood with a mixture of concentrated sulphuric and nitric acid is anything but pleasant considering the large amount of the acid mixture which has to be evaporated. Yet, we are not sure whether the precipitation of the protein with trichloracetic acid is correct since we are totally unaware of the amount of the metals which may be combined organically. We therefore modified the oxidation method so as to make it less objectionable and more convenient. We proceed as follows:

We add slowly and at short intervals about 75 c.c. of a mixture of 100 c.c. of fuming nitric acid and 15 c.c. of concentrated sulphuric acid. (Sp. Gr. 1.84.) After every addition there is a violent reaction with copious foaming. As a precaution against overflowing, we use a 1000 c.c. flask and wait after each addition a few minutes until the reaction subsides. It is quite safe to add 20 c.c. at a time, proceeding slowly, adding and shaking the flask while adding. Cooling is not necessary. After all the acid has been added, driving off of the latter is immediately started. This is easily accomplished when the flask in which the oxidation has been carried out has a ground tubulated (single tube) glass stopper with the outside part of the tube bent under a sharp angle, and the inside about ½″ long. The outside part of the tube is then led into a somewhat larger tube and the connection made air tight with a piece of rubber tubing. If the tube of the glass stopper reaches far enough into the larger tube (about 3″), there is no chance for the hot acid fumes to eat through the rubber. The larger tube passes through the hole of a double perforated cork stopper into a bottle containing 500 c.c. of water. The tube ends at least 2″ above the surface of the liquid, care being taken that throughout the whole reaction it remains outside of the water. The tube which passes through the other hole is bent under a convenient angle and led outside. The same bottle can be used to absorb the excess acid from more than one flask where oxidation is going on, provided a flask with a sufficiently large mouth is chosen.

The concentration of the acid mixture is carried on in the same flask where the oxidation has taken place. The boiling off is stopped when the

volume of the liquid is no more than 5 or 6 c.c. The further treatment will be described later.

Briefly stated our method is as follows:

Calcium and magnesium are precipitated and weighed as stearates (more correctly as a mixture of stearates and palmitates). The latter are then dissolved in N/100 sulphuric acid, the precipitated stearic acid filtered off and the excess of the sulphuric acid titrated with N/100 sodium hydroxide. The total amount of the stearates and that of the stearic acid contained in both of them being known, the respective amounts of calcium and magnesium can be calculated.

We tried to avoid the re-dissolving of the metal bases by titrating the unchanged amount of ammonium stearate and thus determining the quantity of stearic acid which went into reaction. We found the end point to be too vague.

The metals can also be determined by dissolving the stearates in diluted sulphuric acid, precipitating the calcium as oxalate and determining the quantity of the latter by titration with N/100 potassium permanganate. But this alternative offers no visible advantage over our method so far as accuracy is concerned and the additional work which it involves is certainly not an asset.

The following solutions are necessary for carrying out the analysis:

1. N/100 ammonium stearate in an excess of stearic acid. Dissolve 5.5 gm. of stearic acid in as little cold absolute alcohol as possible. Gradually pour the solution into a 1000 c.c. measuring flask to which have been previously added 100 c.c. N/10 ammonium hydroxide and 400 c.c. of water. Shake well after every addition, then fill up to the mark and shake well. Most of the free stearic acid will be found after awhile floating on the top of the liquid. The liquid must be shaken every time a portion of it is to be withdrawn. The excess of stearic acid is necessary in order to prevent the hydrolysis of the calcium and magnesium stearates.

2. Saturated stearic acid solution. Dissolve 1 gm. of stearic acid in as little absolute alcohol as possible. Pour slowly into 1000 c.c. of distilled water, shaking well after every addition; then filter off the undissolved stearic acid.

3. N/100 sodium hydroxide.

4. N/100 suphuric acid.

5. Hydrochloric acid solution of benzidin hydrochloride. Take 9.32 gm. of benzidin hydrochloride (represents 6.7 gm. of the base), add 25 c.c. hydrochloric acid (Sp. Gr. 1.12), transfer the liquid to a 1000 c.c. measuring flask, rinse the beaker with water and fill the flask up to the mark. One c.c. of this solution corresponds to 3.57 mg. of sulphuric acid.

We will now give a detailed description of our method.

Twenty-five c.c. of whole "oxalated" blood (citric acid is to be used) are oxidized in the previously described way, the solution concentrated, transferred to a platinum dish or large crucible which is placed in an inclined position on a triangle with the cover inclined against the upper

edge of the dish and resting on the triangle. The flame is directed against the cover. Fumes of sulphuric acid begin to come off. The flame is then shifted towards the base which is heated to a dull red heat until the white fumes are no longer visible. After the mass in the crucible has been allowed to cool down, a little ammonium carbonate is added which will convert the excess of sulphuric acid into ammonium sulphate which is volatile. The residue is dissolved in as little water as possible, the filtered solution transferred to a beaker of 100 c.c. capacity, the crucible rinsed with several smooth portions of water, care being taken that the total volume of water should not be more than 25 c.c. We now add 25 c.c. of the above described ammonium stearate solution shaking the latter well before addition, filter off the precipitated stearates into a dry weighed Gooch crucible, wash the beaker and precipitate with small portions of the aqueous stearic acid solution described previously, altogether 175 c.c., dry at 90° C., wash with about 50 c.c. of petrol ether in small portions, dry at 120-130° C. until weight becomes constant, and weigh.

The stearates are now dissolved on the Gooch crucible in exactly 10 c.c. of N/100 sulphuric acid and the residue washed in about 40 c.c. of distilled water, using 10 c.c. at a time. The clear solution is then titrated with N/100 sodium hydroxide using methyl orange as indicator. The number of c.c. of N/100 sulphuric acid found is deducted from that which was originally added to the stearates and is equivalent to the amount of stearic acid contained in the stearates.

Calculation.—In the first place, we have to determine the molecular weight of the stearic acid used as ammonium salt for the precipitation of the earth alkalis. There are several brands of stearic acid on the market, many of them labelled C.P. But, it seems that there is no clear conception in the minds of their manufacturers as to what is a chemically pure stearic acid. The molecular weight of almost all the brands of stearic acid (with the exception of one) is 269-272, indicating that they are probably mixtures of stearic acid with palmitic acid. (Molecular weight of stearic acid is 284.4, that of palmitic acid is 256.3.) The melting point is 50-53° C. (Melting point of stearic acid is 69.32° C. and that of palmitic acid is 63.4° C.) The only stearic acid which has the correct molecular weight and melting point is the one manufactured by the Eastman Kodak Co., Rochester, N. Y. But it is too expensive and any other acid, labelled C.P. can be used instead if the molecular weight is determined correctly.

This is done in the following way:

Five grams of stearic acid are weighed out accurately on the analytical balance and dissolved in about 50 c.c. of absolute alcohol, which has been previously distilled over calcium oxide in order to render it absolutely neutral. The solution is transferred to a 100 c.c. measuring flask, the beaker is rinsed out several times with small portions of warm absolute alcohol which are transferred to the flask.

The latter is allowed to cool, whereupon it is filled up to the mark with absolute alcohol and shaken very thoroughly. Ten c.c. are titrated with

N/10 sodium hydroxide using phenolphthalein as indicator. After 10 c.c. of N/10 sodium hydroxide have been added and the end point is not yet reached, it is necessary to add about 10 c.c. of alcohol to prevent hydrolysis of the formed stearate.

Let us suppose that 19 c.c. of N/10 sodium hydroxide were used up to neutralize the 10 c.c. of the alcoholic solution of stearic acid. These 10 c.c. contain 0.5 gm. of stearic acid and are equivalent to 19 c.c. of N/10 sodium hydroxide or to 0.076 gm. sodium hydroxide. We have the following formula:

76 mg. NaOH are equivalent to 500 mg. Stearic acid
40 " " " " " x " " "

$$X = \frac{500 \times 40}{76} = 270.5, \text{ molecular wt.}$$

Let the total weight of the precipitated stearates be A, the number of c.c. of N/100 sulphuric acid used up to decompose the stearates be V, (V is the difference between 10, the number of c.c. of N/10 sulphuric acid added to decompose the stearates, and the number of c.c. of N/100 sodium hydroxide used up to neutralize the excess of the sulphuric acid). V thus represents the number of N/100 stearic acid which were used up to precipitate the stearates.

1 c.c. N/100 stearic acid = 2.7 mgs. S.A.
V c.c. N/100 stearic acid = V × 2.7

Let the amount of calcium stearate be X, that of magnesium stearate is then A-X. The molecular weight of calcium stearate is 579, that of magnesium stearate is 563.

$$\frac{\text{Stearic acid}}{\text{Calcium Stearate}} = \frac{541}{579}$$

$$\frac{\text{Stearic acid}}{\text{Magnesium stearate}} = \frac{541}{563}$$

$$\frac{541}{579} \times X + \frac{541\ (A-X)}{563} = 2.7 \times V$$

$$541 \times 563 \times X + 541 \times 579 \times A - 541 \times 579 \times X = 579 \times 563 \times 2.7 \times V$$

$$X = \frac{541 \times 579 \times A - 579 \times 563 \times 2.7 \times V}{541\ (579 - 563)} = 541\ (579 - 563)$$

Next follows the determination of potassum and sodium. The filtrate from the precipitated stearate is evaporated to complete dryness and the residue heated gently in order to destroy the ammonium stearate. It is then taken up in a little hot water, transferred to a weighed crucible, the water is evaporated on the water-bath and the crucible dried and weighed, the drying and weighing being repeated until there is constant weight. Having thus determined the total amount of the alkali sulphates, we now determine the amount of the sulphuric acid present in both of them.

To the solution of the sulphates are added 25 c.c. of the previously described benzidin hydrochloride solution, stirring vigorously. The benzidin sulphate is precipitated and filtered off after ten minutes, using gentle suction. The last portions of the precipitate are transferred to the filter

with the aid of small portions of the clear filtrate and then the beaker and the precipitate are washed with 20 c.c. of cold water, added in several portions. The precipitate is transferred with the aid of 50 c.c. water to an Erlenmeyer flask and the latter shaken until an homogeneous mass is obtained. The flask is kept closed during shaking, closed with a rubber stopper which is later removed and rinsed off with water. A drop of phenolphthalein is added, the water heated to about 50° C. and titrated with N/20 sodium hydroxide. When the end point is nearly reached, the liquid is boiled for five minutes and the titration is then finished.

Calculation.—The calculation is carried on along the same line as that of the earth alkalis.

Given: Total amount of sulphates = A
No. of c. c. of N/20 NaOH used in titration = V
Amount of K_2SO_4 = X
Amount of Na_2SO_4 = A — X

The amount of sulphuric acid present in both sulphates is equal to:

$$\frac{H_2SO_4}{K_2SO_4} \times X + \frac{H_2SO_4}{Na_2SO_4} \times (A-X) =$$

$$0.563 \times X + 0.696\ (A - X) = 4.9 \times V \text{ mgs.}$$

$$X = \frac{696\ A - 4900\ V}{133}$$

We wish to thank Dr. Max Kahn for suggesting this problem, and for his advice during the course of the work.

A MODIFICATION OF THE FOLIN-WU BLOOD SUGAR METHOD*

By Sergius Morgulis, A. C. Edwards and Elizabeth A. Leggett,
Omaha, Neb.

RECENTLY S. R. Benedict published a new method for the determination of uric acid in blood filtrates, employing an arseno-tungstate solution. We found that by a slight modification we could adapt this uric acid reagent for blood sugar determination. By this new reagent we could obtain values identical with those yielded by the Folin-Wu method, but the color developed with our reagent is about 1.7 times as deep and is of a quality of blue more easily read in the colorimeter.

In searching for a reagent which would give more color than the Folin-Wu reagent we were prompted by a desire to find a means of determining sugar in a single drop of blood (0.05 c.c.). Although we were successful in that by this new reagent a strong color is obtained even with such small amounts of blood, the variations in the results—in our experience, at any rate—were so great as to practically nullify the significance of blood sugar determinations

*From the Department of Biochemistry, University of Nebraska, College of Medicine, Omaha, Neb.
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made on single drops. We are aware of the fact that good success has been recorded by others who measured the sugar in drops of blood by the Folin-Wu method. In our judgment the extra care which must be exercised in working with minute quantities, and the refinement of a technic suitable for such microchemical work practically preclude the use of these methods in clinical procedure as a general routine. There have been, of course, also so-called "drop methods" described, but the amount required for a determination is so great (about 0.5 c.c.) that we find it much simpler to obtain the blood for analyses from a vein by means of a needle in the usual way.

The blood filtrates are prepared exactly as outlined by Folin-Wu in their excellent system of blood analysis. Depending on the amount of sugar expected, one or two c.c. of the filtrate are boiled with two c.c. of the alkaline copper sulphate for six minutes in Folin's sugar tubes. So far, therefore, the procedure is precisely the same as that of Folin-Wu. If the blood contains more than 0.15 per cent sugar we take only one c.c. of the filtrate.

As soon as the tubes are removed from the boiling water-bath we add to it one c.c. of our modified sugar reagent. This is prepared as follows: 100 grams of sodium tungstate is dissolved in about 600 c.c. of water, then 50 grams of pure arsenic pentoxide is added to the solution. When the material is completely dissolved, 25 c.c. of 85 per cent phosphoric acid and 20 c.c. of concentrated hydrochloric acid are added and the mixture is boiled for twenty minutes. After cooling, it is made up to one liter. This is the uric acid reagent made according to Benedict's directions. The sugar reagent is prepared from this by adding 8 c.c. of concentrated hydrochloric acid for 100 c.c. of the arsenophosphotungstate reagent.

Although the sugar reagent can be prepared satisfactorily with smaller amounts of hydrochloric acid, we prefer to use the stronger acid mixture, as it dissolves more easily and quickly the cuprous oxide resulting from the reduction of $CuSO_4$ by the sugar. Increasing the proportion of acid beyond 8 parts for 100 parts of the uric acid reagent is to be avoided as with the stronger acid mixtures weaker colors are obtained.

After the proper amount of the acidified reagent has been added the tubes are cooled by submerging in cold water for three minutes. One c.c. of an exact 10 per cent sodium hydroxide is now added and the contents of the tube diluted with water after standing for five minutes. We generally match the color five minutes after dilution.

The results by our modified procedure agreed with those obtained on the same material by the Folin-Wu method. The comparisons were made with known sugar solutions and with blood filtrates. For a long time all blood sugar determinations in this laboratory were made by both methods simultaneously and invariably with the same result. A few examples will suffice to demonstrate the agreement between the two methods:

Folin-Wu Method	Modified Method
21.4	21.4
17.6	17.9
18.3	18.4

The uric acid content of the blood does not interfere with the sugar reaction. We applied the modified method both to artificial mixtures and to blood filtrates containing variable amounts of uric acid, but in no instance did we find that the sugar values as determined by either the Folin-Wu or by our method were affected.

A sugar determination was made on a blood filtrate and by both methods was found to contain 109 mg. per 100 c.c. of blood. The filtrates were then diluted with a uric acid solution 1 to 9 and 2 to 8 parts of the filtrate. We thus obtained mixtures corresponding to bloods containing 5 and 10 mg. of uric acid per 100 c.c. blood in excess over the amount present originally in that blood. Sugar determinations were again made by the two methods. In the first mixture we found 98.5 mg. of sugar and in the second 87.7 mg. The calculated amount was 98.1 mg. ($109 \times 0.9 = 98.1$) and 87.2 mg. ($109 \times 0.8 = 87.2$) respectively.

It is obvious, therefore, that by our modified method results are obtained which check with those by the Folin-Wu method and that both are unaffected by the initial amount of uric acid in blood.

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A PLEA FOR A STANDARDIZED METHOD OF REPORTING WASSERMANN TESTS*

By Robert A. Kilduffe, A.M., M.D., Pittsburgh, Pa.

A GREAT deal of effort has been expended upon the various factors influencing the Wassermann reaction and, in the endeavor to enhance the delicacy and specificity of the reaction a multiplicity of technical and manipulative refinements has been introduced.

As a result, practically the only vestige of the original test remaining is the name of its sponsor.

It is natural, under these circumstances, that differing results are at times obtained by different workers using methods which possess a wide potential range of delicacy and specificity and that, particularly in the borderline case where the reaction has often a vital significance, the clinician is sometimes puzzled by the reports made to him.

This fact has led to the recognition of the necessity for the development of a standard method of performing the test which, when generally adopted, may be expected to produce a uniformity of results.

Recent studies along this line give hope that the evolution of a method suitable from the standpoint of delicacy and specificity for standard adop-

*From the Laboratories of The Pittsburgh Hospital.
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tion has been achieved. Its general adoption, however, requires time. Confidence in the method must be achieved by a large number of comparative and analytical tests; manipulative dexterity must be acquired, and, above all, personal preferences must be overcome before the method comes into general use.

In the meantime, it would seem advisable to consider the wisdom of an attempt at uniformity in several other phases of the serologic diagnosis of syphilis.

1. Clinicians in general should be at least sufficiently familiar with the test and the factors influencing it to correlate and interpret the findings in conjunction with the other findings of a particular case.

2. The Wassermann test should not be made, as it is in a large proportion of cases, the sole and main avenue of investigation as to the presence or absence of syphilis.

3. The diagnosis of syphilis should not be looked upon as the sole prerogative of either serologist or clinician, but rather as a problem requiring the joint efforts of both, working, not separately, but together.

4. The clinician should insist upon some standard by which to judge the competence and ability of the worker from whom he receives a Wassermann report; he should be able to know if his training has been complete, thorough, and adequate, or if it comprises simply a rule of thumb method of putting a little of "this" in a tube and some of "that" followed by certain other manipulative details. It should be known whether the worker's ability is merely manipulative or whether it includes the ability to interpret as well as to make the test.

5. The clinician should demand something more than a simple "positive" or "negative" report.

In connection with this last, in order that comparisons may be made of various tests: that the relative value and reliability of reports by various methods can be estimated, it seems that a clinician working with a serologist in whose ability he has confidence—and still more so when the serologist is an unknown quantity—is entitled to more information than he often receives.

Different methods vary in reliability and delicacy and, in borderline, weakly-reacting cases, the results are appreciably influenced by variations of technic, and variations in reports on the same case by different workers may be, at times, accounted for by technical differences of method.

For many reasons, therefore, it seems advisable to suggest that a Wassermann report consist of something more than a mere statement of the result arrived at and that some standard, uniform method of submitting reports be agreed upon.

A complete report might rightly include:

(a) The serum dose, because this factor has been shown to influence the character of the results obtained.

(b) The antigens used, because various antigens are very decidedly variant in delicacy.

(c) The result obtained with each antigen, separately.

(d) Possibly, until the adoption of a standard method, the character of the incubation: heat, ice-box or ice-water-bath, etc.

(e) The serologist's interpretation in full of the reaction as a whole.

Such a report permits of more accurate interpretation in doubtful cases and has many advantages. If the worker does not happen to use a cholesterinized antigen, for example, his negative reports on cases under treatment are of less value than if this fact is not known. If his serum dosage is too minute, (drops), his reaction is not apt to be sufficiently delicate to detect only traces of reagin. When reports from two laboratories disagree the answer, at times, may be found in a variation of technic, etc., and so on; many other advantages of a complete, detailed report can be thought of.

A justifiable suggestion, also, is that in cases where clinical and laboratory findings disagree or leave an element of doubt, the furnishing of the clinical data to the serologist will not infrequently be of assistance in the final interpretation of the reaction or help to indicate methods through which a diagnosis can be made.

BLOOD COUNTS WITH OXALATED BLOOD COMPARED WITH ORDINARY COUNTS*

By Alvin G. Foord, M.S., Chicago, Ill.

WHILE doing routine complete blood counts and Wassermann tests on the patients of the Colfax School for the Tuberculous and also handling counts for physicians who had to go considerable distances to obtain blood for counts, the need was seen for a simple way of transporting blood to the laboratory and a way whereby the puncture of the ear might be avoided, inasmuch as the patient is "stuck" once when the Wassermann sample is being taken. Counts directly on the venous blood are not practical for the hemoglobin estimation is not accurate by the Dare or Tallquist methods, and also because the blood clots as a rule before all the necessary procedures can be done. Hence the counts were made to see whether the counting of oxalated blood would be reliable. Miss N. Yarbrough[1] in a report on a limited number of cases claimed that the counts on oxalated blood were practically the same as on blood drawn from the ear or finger, and that the counts were accurate if the blood was kept as long as 3 days. My findings were not as favorable as hers as Table I will show. The method used was to simply eject 1 c.c. of blood from the syringe used in taking the Wassermann sample into a ½ dram vial containing a little powdered potassium oxalate, shaking thoroughly, after which the blood was taken to the laboratory, shaken again, and then counted (in most cases in from ½ to 3 hours after drawing) using the same pipette as was used in making the control test from

*From the Laboratory of the Colfax School for the Tuberculous, Colfax, Calif.
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TABLE I

		EAR BLOOD	FRESH OXAL. BL.	24 HR. OXAL. BL.	FRESH VEN. BL.		POLYS.	L. L. & M.	S. L.	MISC.	COMMENTS
Non-Paraffined Tubes	34 cases	13,300	12,380	10,850	.....	Ear Bl.	72.3	6.3	19.9	1.5	7 out of 26 slides worthless.
						Oxal. Bl.	71.3	6.7	21.0	1.0	
Paraffined Tubes	12 cases	9,960	9,960	9,200		Ear Bl.	71.1	6.6	19.4	2.9	3 out of 12 slides worthless.
	5 cases	11,760	11,340		11,440	Oxal. Bl.	68.9	6.3	22.2	2.6	

the ear. The blood was not kept in the ice box except in the summer. In the spring the vials were kept in a cool spot in the laboratory at about 10 or 15° C.

The control ear blood counts were conducted in the usual way, the pipettes being filled and then closed on the ends with rubber bands. The differential counts were made from smears made on slides, and not by the cover slip method. The blood for these counts was drawn a minute or two before the blood from the vein. Hemoglobin determinations were by the Tallquist scale, and were 10 to 15 per cent lower than the Dare method.

Since the oxalated blood saved for counting only half filled the 2 c.c. vials considerable foam resulted on shaking, and drops of blood adhered to the sides of the vial here and there, which may explain a part of the discrepancies in the table. To keep the blood from adhering to the glass, some of the vials were lined with paraffin. A more even mixing could no doubt be accomplished by nearly filling the vials and having a glass bead in the vial to facilitate the mixing, and also the cells would not be broken up as much. Further experiments will be done along this line.

A few counts were made on venous blood as soon as drawn to compare with the oxalate counts and with the ear blood counts. The results follow:

COMMENTS AND CONCLUSIONS

The hemoglobin estimations were in accord in all cases, the red count as a rule slightly lower with the oxalated blood, and the white counts on the oxalated blood were generally a little lower than those from the ear. The difference would hardly be appreciable clinically. It should also be noted that the counts on the fresh venous blood were about the same as on the oxalated blood, and a trifle lower than the ear counts. The oxalate counts are farther off the longer the blood has stood, few being reliable after 24 hours. The reds on the stained smear were poorly stained and were as a rule somewhat broken or disrupted so that study of the morphology was not possible except in a few cells. The chief objection against counting oxalated blood is the finding that so many white cells are broken up, especially the polymorphs, so that the polymorph count is as a rule lower than it should be. Too many slides are absolutely worthless, for the method as conducted to supplant the counting of capillary blood from the ear or finger. The total counts are fairly reliable, but the differential counts are not. However, it seems to me that if the paraffined vials are practically filled with blood so that the air does not make foam which tends to break up the cells, the counts should be quite reliable, especially if the blood is put on ice as soon as possible. However, I doubt whether blood will be satisfactory if it has to be sent through the mails on account of heat it is subjected to in postoffices and mail cars.

The author wishes to acknowledge his indebtedness to Dr. R. A. Peers, whose aid and encouragement made this work possible.

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LOSS OF SUGAR IN OXALATED BLOOD*

By Dora E. Birchard, B.A., Los Angeles, Calif.

IT IS an accepted fact that the sugar content of oxalated blood diminishes on long standing. It is stated, however, that the blood should be kept on ice, and "analyses preferably made on the day of withdrawal."[1] Watson and White[2] have shown that ox blood does not lose materially in sugar in 24 hours, even at room temperature. It was, however, the experience of this laboratory that specimens of oxalated blood in which the protein was not removed within an hour or two after being drawn, showed abnormally low sugar content, even those kept for only 6 or 8 hours on ice. In the case of specimens which had to come from any distance by mail, and under varying temperature conditions, the sugar findings were often so low as to be of no value.

Two possible ways of correcting this difficulty present themselves: If the drop in sugar content is a uniform one with respect to time, a certain factor might be found by which the actual findings could be corrected according to the length of time the blood had stood. On the other hand, some way might be found to inhibit this break-down of blood sugar during the time that must often elapse between the taking of the blood and the actual determination.

Denis[3] suggests the addition of a few drops of commercial formaldehyde to the oxalated blood. This is all right for sugar determinations only, though great care must be exercised to avoid any excess of formaldehyde. In cases where a number of different determinations are to be made on the same filtrate, the presence of formaldehyde tends to interfere with these, particularly the nitrogen determinations. At any rate, if the desired effect can be accomplished without this addition, so much the better. The answer here is the protein-free filtrate, provided the loss of sugar takes place in the whole blood and not in this filtrate.

There are then, these two questions to be answered by the experimental evidence below:

(1) Does the decrease in blood sugar occur as a uniform drop so that a definite "per cent loss per hour" factor can be established, and if so, what is this factor?

(2) Does the protein-free filtrate if made at once, maintain its sugar content at a constant figure, and if so, for how long?

*From the Clinical Laboratories of Drs. Grant and Talbott, Los Angeles, California.
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METHODS

The Folin-Wu[4] method of blood sugar determination was the one used. A portion of each specimen was deproteinized by the tungstic acid method, immediately after being drawn. The sugar content of the filtrate thus obtained was taken as the standard for that specimen, that is, 100 per cent in

TABLE I

LOSS IN BLOOD SUGAR IN 24 HOURS IN THE SAME SPECIMEN

SPEC. NO.	MILLIGRAMS OF SUGAR PER 100 C.C. OF BLOOD				PER CENT LOSS		
	AT ONCE	3 HRS.	6 HRS.	24 HRS.	3 HRS.	6 HRS.	24 HRS.
1	157	119	99	97	17.0%	36.9%	38.1%
2	111	78	67	63	*29.9	39.6	43.2
3	129	118	105	85	8.5	18.6	34.1
4	108	94	88	72	12.9	18.5	33.3
5	98	84	80	75	14.2	18.3	23.4
6	125	110	104	93	12.0	16.8	25.6
7	164	138	100	96	15.8	39.6	41.4
8	110	101	91	84	8.1	17.2	23.6
9	103	91	75	68	11.6	27.1	33.9
10	75	67	53	50	10.6	29.3	33.3
				Average	12.3%	26.19%	32.99%

*This figure has been omitted from the 3 hour average because of its very wide variation from the others of its period.

TABLE II

SHOWING THE VARIATION IN THE LOSS OF BLOOD SUGAR IN 3, 6 AND 24 HOURS

NO.	PER CENT LOSS		
	3 HRS.	6 HRS.	24 HRS.
1	17.0%	39.6%	46.1%
2	16.2	39.6	43.2
3	15.8	36.9	42.2
4	15.7	30.5	41.4
5	14.2	29.3	38.1
6	12.9	28.0	36.0
7	12.4	27.1	34.1
8	12.3	22.0	34.1
9	12.0	18.9	33.9
10	11.6	18.6	33.8
11	11.5	18.5	33.3
12	11.2	18.3	33.3
13	10.9	17.4	26.2
14	10.6	17.2	25.9
15	10.4	17.0	25.6
16	8.5	16.9	25.0
17	8.1	16.8	23.6
18	7.9	14.8	23.4
19	7.4	14.4	22.2
20	5.0	14.2	19.0
Highest	17.0%	39.6%	46.1%
Lowest	5.0%	14.2%	19.0%
Average	11.5%	22.8%	31.9%

calculating the per cent loss. After keeping the oxalated blood on ice for 3, 6, and 24 hours, respectively, a new filtrate was prepared for each interval, and the sugar content recorded and compared with the original finding.

Twenty specimens of human blood were tested after 3 hours, twenty after 6 hours, and twenty after 24 hours. In ten of these, the 3, 6, and 24

hour determinations were made in succession on the same specimens of blood. (Table I.) Most of the specimens had a normal sugar content, only two of this series containing over 130 mg. per 100 c.c. of blood.

Table II represents in a condensed form, all the findings of the entire experiment. The loss percentages of the ten specimens of Table I are here, besides others (cases where it was not possible to do more than one or sometimes two intervals on the same specimen). The three columns are not for parallel specimens therefore, but are simply numbered from 1 to 20 for convenience in reading the table.

TABLE III

SUGAR CONTENTS OF BLOOD FILTRATES MADE AT ONCE FROM FRESHLY DRAWN BLOOD, AND KEPT ON ICE FOR 24 HOURS

SPEC. NO.	MILLIGRAMS OF SUGAR PER 100 C.C. OF BLOOD		PER CENT LOSS IN FILTRATE	PER CENT LOSS IN WHOLE BLOOD (SAME SPECIMEN)
	AT ONCE	24 HRS.	24 HRS.	24 HRS.
1	104	104	0%	25.9%
2	145	146	0	26.2
3	154	148	3.8	42.2
4	158	158	0	34.1
5	97	97	0	39.1
6	110	101	8.1	30.0
7	110	109	0	25.0
8	130	130	0	33.8
9	105	108	0	19.0
10	135	137	0	22.2
	AT ONCE	18 HRS.	18 HRS.	18 HRS.
11	103	104	0	32.0
12	80	80	0	22.5
	AT ONCE	36 HRS.	36 HRS.	36 HRS.
13	460	460	0	21.0

It will be seen from these tables that while it is possible to strike an average, yet this average will be rather widely at variance with the upper and lower limits of its series, and that these upper and lower limits of the three series overlap one another to a marked degree. It is plain therefore, that no absolutely definite factor can be derived, although we may conclude with reason, that the blood does lose about 12 per cent in the first 3 hours, about 22 per cent in the first 6 hours, and about 32 per cent in the first 24 hours. This knowledge may be of considerable value if a sugar determination is being made on blood which has unavoidably stood for some time. If it should be desirable, for the purpose of checking results, to repeat a sugar determination after several hours have elapsed, it must be remembered that the second finding should be approximately 12 per cent lower than the first, if the time between them has been 3 hours, or 22 per cent lower for 6 hours, etc.

For the second part of the experiment, the original filtrate was kept on ice and sugar determinations made on this after 24 hours (in two cases after 18 hours, and in one case after 36 hours). At the same time a new filtrate from the same specimen of blood was made and analyzed, and the

sugar contents of these two filtrates were compared with the original finding and with each other. (Table III.)

In all but two cases there was no decrease in the sugar values in the filtrates made from fresh blood, even though these filtrates were kept for 24 hours, or even 36 hours. A further extension of the time was not carried out. For the two exceptions we have no explanation to offer as yet, other than individual variation; but the risk of loss of sugar in the filtrate is so slight as compared with the certainty of its loss in the whole blood as to be almost negligible.

Herein then, lies the most feasible solution to the problem. The blood should be deproteinized immediately, or within an hour after its removal from the patient. After this treatment it may stand several hours or a day or longer without appreciable loss. If it is not to be kept on ice a few drops of xylene should be added. The removal of the protein requires little time, and only two reagents, the 10 per cent sodium tungstate and ⅔ N sulfuric acid. These should be kept on hand in the laboratory which first receives the blood (if it is to be sent elsewhere for analysis), and the protein removed at once. The clear filtrate may then be sent on, or kept until a convenient time, with the above precautions.

SUMMARY

1. The sugar content of oxalated blood decreases with a fair degree of regularity, though no absolute factor for this decrease, hour by hour, can be established. Approximate figures, however, may be of considerable value, and these, as determined by the experiments, are as follows:

About 12 per cent for the first 3 hours.
About 22 per cent for the first 6 hours.
About 32 per cent for the first 24 hours.

2. The protein-free filtrate maintains a practically constant sugar percentage, for at least 36 hours, if kept on ice, or with the addition of a few drops of xylene if kept at room temperature.

3. It is important then, that the protein be removed immediately after the blood is drawn. The filtrate may then be kept (preferably on ice) until a convenient time, or may be sent to a distant laboratory for analysis.

I wish to acknowledge with thanks the assistance and valuable advice given by Dr. H. L. White, of this laboratory.

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EDITORIALS

Therapy of Neurosyphilis

SOON after the elaboration of the Wassermann reaction those parasyphilids of Fournier which involved the central nervous system, were shown by spinal fluid examination to be syphilitic in origin. This observation was acclaimed, since the possibility now existed that antisyphilitic treatment might induce cure in tabes and general paresis. Soon, however, it was found that the results did not warrant the high expectations and that in spite of intensive intravenous therapy, the majority of cases of outspoken tabes and general paralysis progressed. It was then assumed that the central nervous tissues could not be reached as satisfactorily by intravenous medication as was the case with other tissues, and a method of direct application of salvarsanized serum into the spinal canal was rapidly developed. Even then many cases progressed with little or no improvement. These failures appear due chiefly to the fact that the average case of neural syphilis does not receive intensive treatment until after irreparable parenchymatous damage has occurred. The most successful treatment should be that which is instituted at a time when central nervous involvement can only be recognized by the positive spinal fluid findings.

There is some diversity of opinion as to what therapeutic procedure may best be followed in obvious nervous system infection. In general, three views are held. First, that intravenous medication alone produces satisfactory results; second, that intravenous medication should be combined with spinal drainage; and third, that the administration of salvarsanized serum intraspinally produces the greatest benefit. A few recent articles have called attention to the possibility of increasing neural damage after intravenous medication alone, by a reduction of the concentration of immune bodies. This suggestion has been comprehensively reviewed in an editorial of the January, 1922, number of this *Journal.*

Dercum has been one of the leading protagonists of spinal fluid drainage as against the administration of salvarsanized serum. He points out that the function of the cerebrospinal fluid is preeminently mechanical and that the fluid exists practically alone for the purpose of suspending the brain and cord in a fluid medium and protecting it against trauma. A brain weighing from forty-five to fifty ounces exerts a pressure of but one ounce on its base, due entirely to the supporting action of the spinal fluid. In its chemical constitution, the spinal fluid corresponds more nearly to pure water than does any other body fluid. It contains inorganic salts similar to those of the blood plasma, a trace of coagulable protein and a very small amount of glucose. It is directly continuous through the aqueduct of the cochlea with the fluid of the labyrinth which is generally conceded to exist for mechanical purposes only.

The fluid is distributed through the ventricles and subarachnoid spaces and apparently has no relationship to the perivascular, pericapillary or perineuronal spaces of the cord and brain. It has its source in the choroidal plexuses and possibly also on the serous surfaces of its containing cavities, and leaves through the subarachnoid villi, entering into the venous sinuses. To a lesser extent it also passes out through the lymph sheaths of the cranial and spinal nerves. Dercum states that intraspinal medication was undertaken on the erroneous theory that the nutrition of the nervous system is carried on through the spinal fluid, and that the injection of medicated serum distributes the arsenic into this nutrient medium, thereby circumventing the hypothetical obstruction to the passage of arsphenamine through the choroid plexus.

Craig and Chaney, who also advocate spinal drainage, doubt whether there is much actual obstruction in the choroidal villi to the passage of arsenic. Thus Sicard and Block recovered arsenic from the spinal fluid in three of eleven cases after intravenous injection of arsphenamine. Ravaut obtained similar results in three of thirty-three cases, Hall in two out of eight and again in four out of seven, and Rieger and Solomon in thirty-eight out of one hundred and twenty-three. It must be borne in mind, however, that while arsenic is thus shown to be present in the spinal fluid following intravenous medication, in the majority of cases it was not found. Camp found a questionable trace of arsenic in one of seventeen cases, Benedict in four out of a large series treated, Engman and his collaborators in none of

four cases, and Hall in a third series examined, found arsenic present in one case and absent in nine.

Rieger and Solomon found that in general those patients consistently showing the larger amounts of arsenic in their fluids made the more rapid improvement.

Craig and Chaney agree with Dercum that intraspinal therapy is unscientific because of the rapidity with which chemicals introduced into the subarachnoid space disappear into the general circulation. Thus Rieger and Solomon reported rapid removal of arsenic from the spinal fluid after intravenous arsphenamine, and Hall reports similar rapid disappearance after intraspinal administration. Other substances, such as phenolsulphonephthalein, enter the blood from the subarachnoid space with equal rapidity, appearing in the urine within from four to ten minutes after subdural injection.

The advocates of spinal drainage do not deny the good results obtained by the Swift-Ellis method but maintain that the benefit derived is due not to the substances introduced but to the preliminary spinal drainage incidentally practiced.

The performance of spinal fluid drainage after intravenous treatment was originally based on the assumption that with reduced intraspinal pressure, the arsphenamine circulating in the blood will diffuse more readily into the subarachnoid space. Dercum claims better results than by the Swift-Ellis method, due he thinks, to the more thorough drainage. Every possible drop of fluid is removed each time. Theoretically, the rapid removal of fluid will produce a relative hyperemia of the cord and brain with resulting improved nutrition to the parts. Dercum draws an analogy to the Bier method of hyperemia as used in surgery. If his theory is true, drainage alone irrespective of intravenous treatment might improve the nutrition of the central nervous system with resultant spontaneous improvement.

The presence of arsphenamine in the spinal fluid according to Dercum, is of no significance and probably plays no part in the good results obtained. The difficulty in syphilitic medication lies not in the failure of passage of the spirocheticide through the choroid plexus into the subarachnoid space, but in its failure to pass through the walls of the capillaries situated in the nervous tissue, into the parenchymal cells where the chief damage takes place. The hyperemia resulting from spinal drainage would increase any such tendency to pass through the capillary walls.

Corbus and his collaborators have elaborated a new method in which they believe that they can produce equally efficient spinal drainage without the performance of lumbar puncture. Their work is based on experiments of Foley, who found that after the intravenous injection of hypertonic salt solution, the pressure of the spinal fluid falls markedly. This fall is accompanied by a diminution in brain bulk rather than by an increase. Foley in studying decompression hernia, found that after intravenous injection of 15 per cent saline solution the spinal fluid was almost entirely absorbed into the general circulation as evidenced by recession of the hernial protrusion. This covered a period of two or three hours. The herniation returned within

from six to seven hours after the injection. The increased osmotic tension of the plasma evidently drew fluid rapidly into the blood stream, from all available sources including the subarachnoid space.

Corbus and his associates give 100 c.c. of hypertonic salt solution intravenously followed in six hours by intravenous injection of neoarsphenamine. Treatments are given weekly. They do not draw didactic conclusions from their work and their tabulated results do not allow comparison with other forms of treatment. They apparently assume that penetration of arsenic into the subarachnoid space is an important factor in treatment and claim for their method that this penetration is greater than in any other form of treatment. They found arsenic in the spinal fluid in 93 per cent of their twenty-eight cases.

Rudolf and Bulmer have made chemical tests for arsenic in the various organs and tissues, after intravenous and intraspinal injection of phenasenamine, and report that after either method practically none can be demonstrated in the central nerve tissues.

Fordyce, who is an ardent advocate of the Swift-Ellis method of treatment, takes vigorous exception to Dercum's conclusions. He points out that Swift and Ellis did not develop their method under the mistaken idea that the nervous system receives its nutrition from the spinal fluid, but rather because intraspinal therapy had been successfully employed in the treatment of cerebrospinal meningitis. Furthermore, they demonstrated clearly that salvarsanized serum possesses definite spirocheticidal properties. Many forms of neurosyphilis have their site in the meninges and are limited to these structures. Meningovasculitis cannot always be differentiated clinically from paresis and in most cases of true paresis there exists at the same time a meningitis. Positive spinal fluid findings in central nervous system syphilis are due usually to meningeal involvement and this can best be treated by the direct application of a spirocheticide. The more pronounced the luetic meningitis, the more marked will be the benefit derived from intraspinal medication. Fordyce states that numerous cases have been entirely cured and many greatly benefited by Swift-Ellis treatment after the failure of those methods so strongly advocated by Dercum and others. Numerous cases of progressing optic atrophy have been permanently arrested by intraspinal medication after the failure of prolonged intravenous treatment.

Fordyce now omits preliminary drainage and introduces the salvarsanized serum under pressure, with better results than when he was using incidental drainage. Syphilis of the nervous system may exist without coincident clinical or serologic evidence of blood stream infection and in certain of these changes, there is no interchange of the products of syphilis between the nervous system and the blood, nor is there penetration of drugs introduced into the blood stream to the central nervous system. Fordyce states that he has confirmed this statement again and again, and that in these cases remedies applied through the ordinary channels are of no avail.

He emphasizes repeatedly that the problem of the future in neurosyphilis is one of prophylaxis wherein the occurrence of neural invasion

must be recognized before the production of permanent parenchymatous damage.

The prevention of late neurologic accidents in syphilis, however, does not depend solely upon a recognition of early invasion. The theory that inadequate treatment predisposes to late nervous involvement has been discussed in some detail by Keidel. Little is known with regard to immunity in syphilis but we have considerable indirect evidence that the human body does develop some degree of immunity against the Treponema pallida. The observation of Colles regarding the apparent immunity of the pregnant mother and that of Profeta, that a "nonsyphilitic" child born of syphilitic parents appears immune, were the first observations based on such a conception. As a rule a syphilitic cannot be superinfected. Pearce and Brown have shown experimentally that insufficient treatment will destroy this immune mechanism and that a second chancre may then be produced even while pathogenic microorganisms persist in the first. They have proposed two "laws," both of which aid in an understanding of late neurosyphilis following inefficient treatment. According to the "law of progression," various tissues of the body are not equally susceptible and reactive to syphilitic infection. Some groups are more susceptible than others, and with a progressing infection there is an orderly sequence from one group to the next. Interference with the course of the infection, as by treatment, may protect some tissues, but unless persisted in may fail to protect tissues higher in the scale. Keidel observes that certain tissues with relatively high resistance to invasion show little defensive reaction after finally becoming involved. If these observations are applied to the central nervous system the latter tissue may be considered relatively insusceptible to invasion with the spirochete, and at the same time unable to satisfactorily develop an immunity reaction when it does become so invaded. Thus the nervous system must depend for its protection upon the presence of immune bodies derived from other tissues. With partial destruction of the treponema, the general immunity reaction becomes less vigorous and the nervous tissues more easily fall prey to the invading parasite.

The second law formulated by Brown and Pearce, "the law of inverse proportions" assumes an inverse quantitative relationship in the intensity of consecutive reactions in syphilitic infection. Thus in the primary sore a defense reaction is set up. If this is vigorous and the chancre is extensive, the later manifestations are likely to be milder if they appear at all. Conversely, with a small, nonreactive chancre, the secondary and tertiary phenomena are usually more extensive. It has long been observed that in secondary syphilis with extensive cutaneous manifestations, central nervous infection is less apt to occur, whereas with but slight cutaneous involvement, later neurosyphilis is more frequent. Treatment which mitigates the severity of the primary and secondary reactions without completely eliminating the infection may predispose to involvement of those more highly resistant tissues such as the central nervous system.

Thus we may use the term accurately when we speak of prophylactic

treatment of neurosyphilis. The efficiency and completeness of treatment of the primary and secondary lesions appears to be of considerable importance in determining later neural invasion. A completely satisfactory standard prophylactic treatment has not been formulated.

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—*W. T. V.*

Aid to Physicians of Ukraine

IN the January issue we published an editorial briefly describing the situation of the physicians in Ukraine. Believing that many of our readers might desire to aid their colleagues in Ukraine, we shall publish an extract from the letter of Vernon Kellogg, of the National Research Council, in reply to our request for the information contained therein.

"It is a very simple matter to send food relief to any individual or group of individuals in the Ukraine. This can be done by buying food remittances of ten dollars each from the American Relief Administration, 42 Broadway. These remittances are also on sale in many banks. By paying ten dollars you get a certificate in which you put the name and address of the individual whom you wish to benefit and then you send this certificate, keeping a tear-off portion of it as receipt, to your beneficiary in the Ukraine through the regular mails. On receipt of the certificate the beneficiary applies at the nearest American Relief Administration warehouse, or sends his certificate in by mail to this warehouse, and receives the food. There is no difficulty at all about the sending and receipt of these certificates, nor about cashing them in food. The American Relief Administration has up to date sold about eleven million dollars worth of these remittances and over 95 per cent of them have been cashed in Russia. I would suggest that as the American Relief Administration's work in Russia is expected to close early in the summer it would be well to get any food remittances, that you care to send, over promptly."—(Signed) Vernon Kellogg.

Erratum

On Page 219 of the January Issue of the Journal, the 18th line should read: "6 × 0.17 G. = 1 G.; Marked depression (part retained)." instead of "died within 12 hours."

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, St. Elizabeth's Hospital, Richmond, Va.)

*Preventive Medicine**

THIS is a book by most competent men. While it is written by Canadians and has its direct application to the Dominion of Canada, it is of equal interest and value to physicians in the United States. The preface gives a picture of the contents of the book. After dwelling upon the start in preventive medicine that has already been made in Canada and in this country, the authors state: "Two further steps are necessary. First, the state should provide a service for those to whom the cost of complete health supervision is prohibitive and such a service should be free to all, rich and poor alike, and no stigma of charity should be attached to its acceptance. This service should include provision for antenatal supervision, home visitation, and infant welfare centers to safeguard the health of infants and little children. Medical, nursing and dental supervision in schools and colleges, and finally, clinics for periodic medical examinations of adults. Thus, health supervision would be provided for all those who chose to take advantage thereof: from infancy to old age. The second important point should be: No compulsion! Let those who pay their taxes and help provide the service offered by the state, arrange for their own private supervision should they so desire. Free education is now provided at the public expense. Many there are, however, who pay school taxes but take no advantage thereof. They prefer to have their children educated in schools not provided at the expense of the state."

Following a statement of the problems and aims of preventive medicine, there is discussed in a most authoritative and practical way the recognition and control of the various infectious diseases. Nearly one-half the volume is taken up very profitably with a discussion of water, milk, foods, deficiency diseases, community sanitation, maternal and infant mortality, school hygiene, public health clinics and centers, air and ventilation, industrial hygiene and public health organization. We can commend the book and its teachings to all those interested in preventive medicine. —*V. C. V.*

*An Introduction to the Practice of Preventive Medicine, by J. G. Fitzgerald, M.D., F.R.S.C., Professor of Hygiene and Preventive Medicine and Director Connaught Antitoxin Laboratories, University of Toronto, Assisted by Peter Gillespie, M.Sc., C.E., M.E.I.C., Professor of Applied Mechanics, University of Toronto and H. M. Lancaster, B.A., Sc., Director Division of Laboratories, Provincial Board of Health, Ontario. St. Louis, C. V. Mosby Company, 1922.